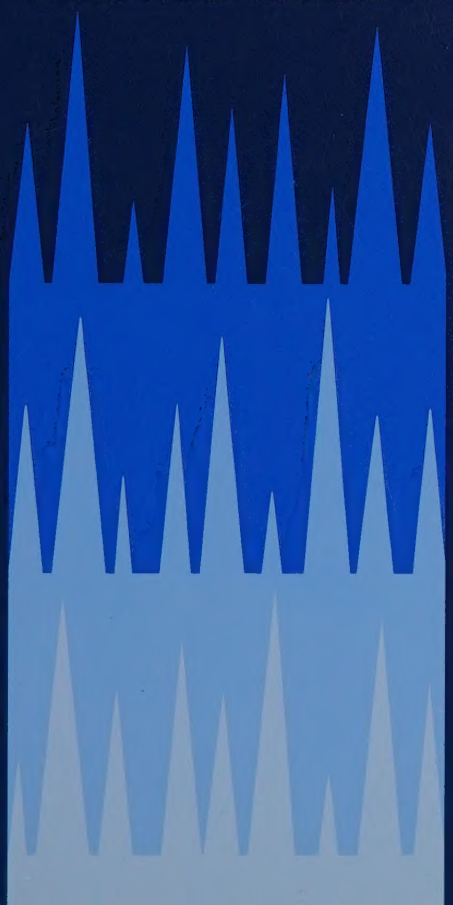
DIONEX

CHROMATOGRAPHY



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INSTALLATION INSTRUCTIONS and TROUBLESHOOTING GUIDE

for the

IONPAC® ICE-AS6 ANALYTICAL COLUMN

(9 x 250 mm, P/N 046023)

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Revision 01
15 March 1993

INITIAL INSPECTION

As soon as you unpack the IonPac® ICE-AS6 Analytical Column, inspect it for shipping damage. Because many carriers limit the claim period to 10 days after the delivery date, DIONEX recommends that you report any shipping damage to the incoming carrier immediately. To request help in assessing shipping damage, or to report a missing or damaged part, contact the DIONEX Regional Office nearest you (see Section 10, "DIONEX Regional Offices").

COLUMN WARRANTY

The 90-day column warranty is in effect from the day the IonPac® ICE-AS6 Analytical Column is shipped from DIONEX. After verifying that it was not damaged in transit, install the column in the system and test it by making several injections of the standard used to obtain the test chromatogram. The test chromatogram is shipped with the column. Contact the nearest DIONEX Regional Office (see Section 10, "DIONEX Regional Offices") if the performance of the column is unsatisfactory.

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SECTION 1 - INTRODUCTION TO IONPAC ICE-AS6 ION EXCLUSION CHROMATOGRAPHY

The IonPac ICE-AS6 is an ion exclusion column designed for efficient separation of low molecular weight aliphatic organic acids including hydroxy- substituted organic acids, aliphatic alcohols and glycols.

The IonPac ICE-AS6 is uses an ion exclusion mechanism, Donnan exclusion (see Figure 1, "The Ion Exclusion Separation"), which allows retention and separation of weakly ionized acids based on differences in pKa's. Strong inorganic acid anions are not retained by the stationary phase and elute in the void volume of the column. In addition to Donnan exclusion, the ICE-AS6 has hydrophobic functional groups within the resin structure which promote adsorption and hydrogen bonding. These two additional retention mechanisms allow resolution of organic acids which are poorly resolved by ion exclusion alone, such as tartrate (pK_1 = 2.82) from citrate (pK_1 = 2.87) and glycolate (pK_1 = 3.63) from lactate (pK_1 = 3.66) from hydroxyisobutyric acid (pK_1 = 3.72).

In the ion exclusion mechanism shown in Figure 1, "The Ion Exclusion Separation", below, the sulfonated surface is a hydrated, negatively charged boundary called a Donnan membrane which is permeable only to neutral molecules. Totally dissociated inorganic acids, such as dilute hydrochloric acid, can not penetrate the membrane due to electrostatic repulsion and are totally excluded from the stationary phase. This volume is called the exclusion volume, Vc. In contrast, neutral molecules can diffuse into the resin pores. The total permeation volume, Vp, for water is observed as a small negative peak.

The undissociated form of a weak carboxylic acid exhibits a greater retention than water due to adsorption to the resin. In general, the higher the pKa of the acid, the greater the retention. The longer the aliphatic carboxylic acid, the longer its retention time. On the ICE-AS6, the adsorption mechanism, in addition to Donnan exclusion, is very significant. The addition of a solvent such as acetonitrile can be used to reduce retention times of highly retained hydrophobic carboxylic acids. Stronger carboxylic acids such as di- and tricarboxylic acids (i.e. oxalic or citric) have low retention and elute between the exclusion and total permeation volumes.

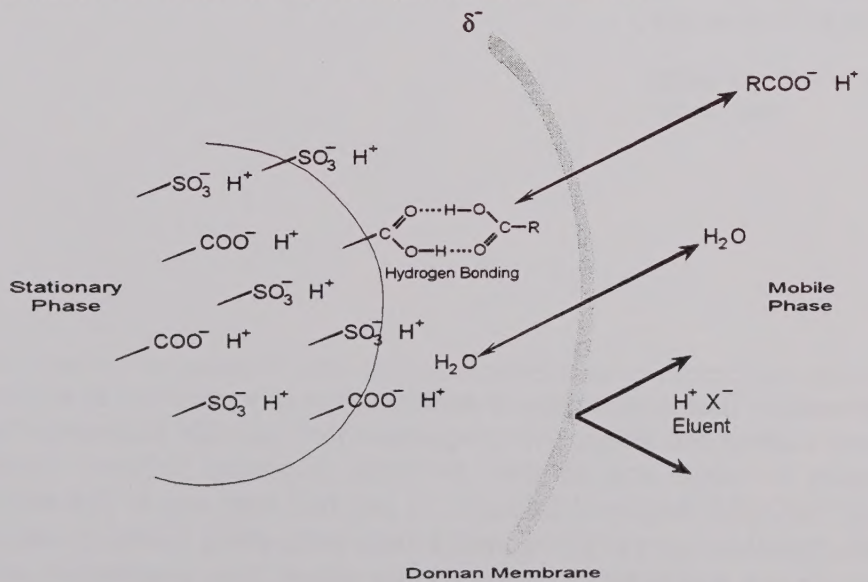


Figure 1

The Ion Exclusion Separation

ICE-AS6 columns are stable between pH 0 - 7 (do not run eluents with cations other than hydronium ion through the ICE-AS6) and are compatible with eluents containing 0 - 20% HPLC solvents such as methanol, isopropanol or acetonitrile.

The ICE-AS6 is composed of a 8 µm cross-linked styrene/acrylate/divinylbenzene resin that is functionalized with sulfonate and carboxylate groups The ion exchange capacity of the 9 x 250 mm analytical column is 27 meq/column. The IonPac ICE-AS6 also has intermediate hydophobicity when compared to the ICE-AS1 and the ICE-AS5. The ICE-AS6 also has intermediate solvent compatibility (20%) when compared to the ICE-AS1 (70%) and the ICE-AS5 (5%).

The ICE-AS6 can be operated at flow rates up to 1.25 mL/min. When setting up the analytical system, observe the special precautions listed in Section 3, "Installation". PEEK (polyetheretherketone) is used to make column hardware. PEEK has excellent chemical resistance to most organic solvents and inorganic solutions. Concentrated sulfuric acid and concentrated nitric acid will attack PEEK. Tetrahydrofuran at concentrations of greater than 20% is not compatible with PEEK systems. The ICE-AS6 Analytical Columns have minimum efficiencies for sulfate of 12,000 plates/column (for acetic acid), under standard operating conditions. The ICE-AS6 operates at a back pressure between 400-600 psi at 1.0 mL/min with the test eluent. However, both columns are capable of operating at back pressures up to 850 psi.

This manual assumes that you are familiar with the installation and operation of the DIONEX Ion Chromatograph (IC) and the Anion-ICE MicroMembrane™ Suppressor. If you do not understand the operation of the system, take the time to familiarize yourself with the various system components before beginning an analysis.

The IonPac ICE-AS6 Analytical Column has 10-32 threaded PEEK end fittings for use with ferrule/bolt liquid line fittings. If your system is otherwise configured, refer to Section 8, "Installation of DIONEX Liquid Line Fittings".

Always remember that assistance is available for any problem that may be encountered during the shipment or operation of DIONEX instrumentation and columns through any of the DIONEX Regional Offices listed in Section 10, "DIONEX Regional Offices". If you feel that any of the sections in these Installation Instructions and Troubleshooting Guide is unclear, incomplete or are not needed, please write down your comments on the Reader's Comments Form at the end of this manual and return the form to us.

SECTION 2 - IONPAC ICE-AS6 EXCLUSION CHROMATOGRAPHY SYSTEM REQUIREMENT SUMMARY

Condition	Operation Summary
Eluent Flow Rate	Typically 1.0 mL/min
Anion MicroMembrane Suppressor	AMMS-ICE (P/N 037107)
Regenerant Flow Rate	Typically 3 - 5 mL/min See Document No. 032661
Injection Loop	10 - 50 µL
System Void Volume	Minimize dead volumes. Switching valves, couplers can be used. The GM-3 Gradient Mixer should be used for gradient analysis on systems other than DX-300 HPLC Pump.
Detectors	VDM-2 Cell (10 µL) (P/N 043113) CDM-2/CDM-3 Cell (P/N 042770) DIONEX Back pressure Regulator 75 psi rating (P/N 039760) or Tubing (see Table 1) Ensure 50-75 psi back pressure. Either the TS-1 or the TS-2 can be used.

SECTION 3 - INSTALLATION

3.1 SYSTEM REQUIREMENTS

The IonPac ICE-AS6 Analytical Column are designed to be run on any DIONEX Ion Chromatograph equipped with suppressed conductivity detection.

3.2 SYSTEM VOID VOLUME

For best performance, all of the tubing installed between the injection valve and detector should be 0.005" (P/N 044221) ID PEEK tubing. 0.010" ID PEEK tubing (P/N 042260) or 0.012" Tefzel tubing (see Section 9, "Important DIONEX Part Numbers") may be used but peak efficiency will be compromised which may also result in decreased peak resolution. Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers. If you need assistance in properly configuring your system contact the DIONEX Regional Office nearest you (see Section 10, "DIONEX Regional Offices").

3.3 THE INJECTION LOOP

Table 1. Smallest Injectable Volumes (µL)

Valve Type Tubing	Using 0.012" ID Tefzel® Tubing	Using 0.007" ID Tefzel Tubing	Using 0.010" ID PEEK Tubing	Using 0.005" ID PEEK
DIONEX BF2 Valve (8 µL Internal Volume) (10 cm Loop)	**15.2**	**10.5**	**13.1**	**9.2**
DIONEX MicroInject Valve (10.5 µL Internal Volume) (14 cm Loop)	**20.5**	**14.0**	**17.6**	**12.2**
Rheodyne Microinjection Valve Model 9126 (0.8 µL Internal Volume) (10 cm Loop)	**8.0**	**3.3**	**5.9**	**2.0**

For most applications on a 4mm analytical system, a 10 - 50 µL injection loop will be sufficient. DIONEX recommends that a 50 µL injection loop be used to avoid overloading the ICE-AS6 Analytical Column.

3.4 ELUENT STORAGE

IonPac ICE-AS6 columns are designed to be used with acid eluent systems and isocratic analysis. Storage under a helium atmosphere ensures contamination free operation and proper pump performance (nitrogen can be used if eluents do not contain solvents). Acidic eluents that contain acetonitrile should be made fresh daily. Acetonitrile slowly hydrolyzes in acidic solutions.

3.5 ANION-ICE MICROMEMBRANE SUPPRESSOR REQUIREMENTS

An Anion-ICE MicroMembrane Suppressor (P/N 037107) should be used instead of an ASRS-I or AMMS-II for ion exclusion applications that require suppressed conductivity detection. It is compatible with all solvents and concentrations with which the systems and columns are compatible. Use DIONEX Cation Regenerant Solution, Tetrabutylammonium hydroxide (TBAOH, P/N 039602). Dilute as required for the example applications.

This manual assumes that you are familiar with the installation and operation of the Ion Chromatograph and Anion-ICE MicroMembrane Suppressor. If you do not understand the operation of your system, take the time to familiarize yourself with the operator's manuals for the products before beginning an analysis. For detailed information on the operation of the Anion-ICE MicroMembrane Suppressor, see Document No. 032661

4.1 GENERAL OPERATING CONDITIONS

The selectivity of the IonPac ICE-AS6 Analytical Column has been designed to separate a broad low molecular weight organic acid anions in less than 20 minutes. The ICE-AS6 columns consist of a cross-linked (8%), microporous, intermediate hydrophobic resin that has been sulfonated. The nature of the cross-linked polymeric structure of the packing material makes ICE-AS6 columns compatible with pH 0 - 7 eluents (see Section 4.1.1, "IonPac ICE-AS6 Operation Precautions) and 0 - 20% organic solvent eluents. The ICE-AS6 can be used with any suppressible ionic eluent that does not exceed the capacity of the Anion-ICE MicroMembrane Suppressor (AMMS-ICE).

4.1.1 IONPAC ICE-AS6 OPERATION PRECAUTIONS

CAUTION

- **20% maximum Solvent Concentration**

  When using solvents over 10%, use a gradient in which 30 minutes is used to achieve the maximum solvent concentration (a step change will cause fronting). When the maximum concentration of solvent is achieved, rinse the column for at least 30 minutes. A step change to the application eluent can then be used to equilibrate the column.

- **DO NOT use sodium hydroxide eluents**
- **Filter and Degas Eluents**
- **Filter Samples**
- **1.25 mL/min Maximum Flow Rate for ICE-AS6**

4.1.2 SOLVENTS

The ICE-AS6 can withstand all common HPLC solvents in a concentration range of 0 - 20%. However, solvents and water should be premixed in concentrations to allow proper mixing by the gradient pump and to minimize outgassing. Ensure that all of the inorganic chemicals are soluble in the highest solvent concentration to be used during the analysis.

When using a solvent in an ionic eluent, column generated back pressures will depend on the solvent used, concentration of the solvent, the ionic strength of the eluent and the flow rate used. As Figure 2 shows, the column back pressure will vary as the composition of water-methanol and water-acetonitrile mixture varies. The practical back pressure limit for the IonPac ICE-AS6 columns is 850 psi.

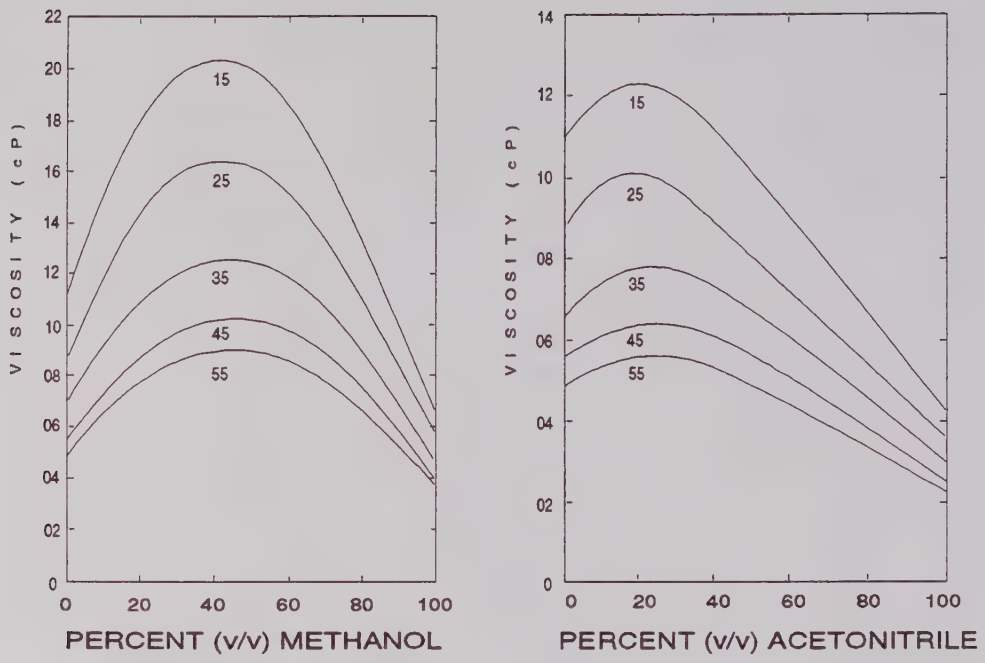


Figure 2

Variation of solvent viscosity with changing temperature and composition of water-solvent mixtures. The composition is expressed as percent (v/v) at 20.5 °C and the viscosity is in centipoise. The temperature at which the viscosity was measured is indicated below each curve.*

Table 2

HPLC Solvents for Use with IonPac ICE-AS6 Columns

Solvent	Maximum Operating Concentration**
Acetonitrile	20%
Methanol	20%
2-Propanol	20%
Tetrahydrofuran	20%

* C. Horvath, Ed., "High Performance Liquid Chromatography. Advances and Perspectives", Vol 2, p. 193-194, New York, 1980.

** See Section 4.1.1, "IonPac ICE-AS6 Operation Precautions"

4.2 CHEMICAL PURITY REQUIREMENTS

Obtaining reliable, consistent and accurate results requires eluents that are free of ionic and spectrophotometric impurities. Chemicals, solvents and deionized water used to prepare eluents must be of the highest purity available. Maintaining low trace impurities and low particle levels in eluents also help to protect your ion exchange columns and system components. DIONEX cannot guarantee proper column performance when the quality of the chemicals, solvents and water used to prepare eluents has been compromised.

4.2.1 INORGANIC CHEMICALS

Reagent Grade inorganic chemicals should always be used to prepare ionic eluents. Whenever possible, inorganic chemicals that meet or surpass the latest American Chemical Society standard for purity should be used. These inorganic chemicals will detail the purity by having an actual lot analysis on each label. The analyses performed in Section 5, "Example Applications", use heptafluorobutyric acid obtained from Fluka Chemie AG.

4.2.2 SOLVENTS

Since solvents used with the IonPac ICE-AS6 columns are added to ionic eluents to modify the ion exclusion process, the solvents used must be free of ionic impurities. However, since most manufacturers of solvents do not test for ionic impurities, it is important that the highest grade of solvents available be used. Currently, several manufacturers are making "ultrahigh" purity solvents that are compatible for HPLC and spectrophotometric applications. These "ultrahigh" purity solvents will usually ensure that your chromatography is not affected by ionic impurities in the solvent. Currently at DIONEX, we have obtained consistent results using High Purity Solvents manufactured by Burdick and Jackson and Optima Solvents by Fisher Scientific.

4.2.3 DEIONIZED WATER

The deionized water used to prepare eluents should be **Type I Reagent Grade Water** with a specific resistance of 17.8 megohm-cm or greater. The deionized water should be free of ionized impurities, organics, microorganisms and particulate matter larger than 0.2 µm. Bottled HPLC-Grade Water (with the exception of Burdick & Jackson) should not be used since most bottled water contains an unacceptable level of ionic impurities.

4.3 ELUENT PREPARATION

4.3.1 ACID ELUENT PREPARATION

The acidic eluents used with the IonPac ICE-AS6 columns are stable and require no special storage. Always prepare eluents with Type I Reagent Grade Water (see Section 4.2.3, "Deionized Water") the has been properly degassed (see Section 4.3.2, "Degassing Deionized Water").

Eluents that contain solvents should be stored in glass eluent bottles pressurized with helium (nitrogen is soluble in solvents).

4.3.2 DEGASSING DEIONIZED WATER

A. Fill glass eluent reservoirs (P/N 042022) with approximately 1 L of deionized water.

B. If you are using a modified EDM-I, disconnect the vent line from each reservoir and remove the barbed fitting from the reservoir cap. Turn on the helium gas supply. Set the SYSTEM ON/OFF toggle switch of the EDM-I to ON and adjust the helium pressure to 10 psi. Set the DEGAS RATE toggle switch to HIGH. Degas the deionized water for 10 minutes before adding 50% sodium hydroxide solution.

C. If you are using a EDM-II, turn on the helium gas supply. Set the SYSTEM ON/OFF toggle switch of the EDM-II to ON. Adjust the helium pressure to 10 psi. Set the RESERVOIR ON/OFF toggle switches of the corresponding eluent reservoirs you are using for the hydroxide eluents to ON. Set the SPARGE/PRESSURE switches to the SPARGE position. Sparge each bottle for 10 minutes before adding hydroxide.

D. Vacuum degassing the deionized water is also an effective way to remove carbon dioxide. Vacuum degas the water by placing the eluent reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump. Vacuum degas the reservoir of water for 5-10 minutes. Cap each bottle and minimize the length of time the bottle is opened to the atmosphere.

E. Use this degassed, Type I Reagent Grade deionized water for the preparation of the specific eluents required to run applications (see Section 5, "Example Applications").

4.4 ELUENTS CONTAINING SOLVENTS

When mixing solvents with water remember to mix solvent with water on a volume to volume basis. If a procedure requires an eluent of 10% acetonitrile, prepare the eluent by adding 100 mL of acetonitrile to an eluent reservoir. Then add 900 mL of deionized water to the acetonitrile in the reservoir. Using this procedure to mix solvents with water will ensure that a consistent true volume/volume eluent is obtained. Premixing water with solvent will minimize the possibility of outgassing.

NOTE

As a rule degass the aqueous component of the eluent and then add the solvent component. Avoid excessive purging or degassing of eluents containing solvents if possible, since it is possible that a volatile solvent can be "boiled" off from the solution.

4.5 ANION SUPPRESSION REGENERANT PREPARATION

The regenerant used with the AMMS-ICE Suppressor when used with the IonPac ICE-AS6 to perform the analyses in Section 5, "Example Applications", is 5 mM tetrabutylammonium hydroxide (TBAOH). Use DIONEX Cation Regenerant Solution (P/N 039602). Dilute 50 mLs of the 0.1 M Cation Regenerant Solution to 1 L with degassed Type I Reagent Grade Water. Prepare several liters of the regenerant.

For a guide to properly adjusting the regenerant flow rate, see Document No. 032661, the Installation Instructions and Troubleshooting Guide for the AMMS-ICE.

The chromatograms in this section were obtained using columns that reproduced the Production test Chromatogram (see Section 5.3, "Production Test Chromatogram") on optimized Ion Chromatographs (see Section 3, "Installation"). Different systems will differ slightly in performance due to slight differences in column sets, system void volumes, liquid sweep-out times of different components and laboratory temperatures.

The IonPac ICE-AS6 may be used isocratically for chromatographing a large number of carboxylic acids.

If your sample or standard contains organic acids, adding chromate (about 10 mg/L) will help stabilize them from bacterial degradation at room temperature.

Before attempting any of the following example applications, take the time to ensure that your system is properly configured.

Ensure that all of the eluents have been made from high purity reagents and deionized water. All water used in the preparation of eluents should be degassed, deionized water. For chemical purity requirements see Section 4.2, "Chemical Purity Requirements".

Run synthetic standards to calibrate and confirm the operation of your system. This column has a very high loading capacity and can handle a large number of dirty samples. If after running complex samples, the ICE-AS6 shows signs of fouling, refer to the column cleanup protocols in Section 7, "Column Care".

5.1 PREPARATION OF ELUENTS

The standard eluent for the example applications presented in this section is 0.4 mM heptafluorobutyric acid. It was prepared from heptafluorobutyric acid (same as perfluorobutyric acid) obtained from FLUKA Chemie AG (P/N 77249). It is >99% (GC) purity with a molecular weight of 214.04 and a density of 1.652. It is supplied in 10.0 mL bottles (16.52 g).

Heptafluorobutyric Acid Stock Solution (0.0772 M):
Dilute the entire contents of one 10.0 mL bottle to 1 L.

Heptafluorobutyric Acid Eluent (0.4 mM):
Dilute 5.2 g of the stock solution (0.0772 M) to 1 L.

If you prefer to work from a 0.100 M stock solution, dilute 21.40 g of the >99% purity heptafluorobutyric acid to 1 L. The eluent can then be made by diluting 4.0 g of the 0.100 M stock solution to 1 L to obtain the 0.4 mM heptafluorobutyric acid eluent. Heptafluorobutyric acid was chosen for minimum background conductivity. However, other 0.4 mM monoprotic acids can be used (see Section 5.5, "Comparison of Different acids used on the IonPac ICE-AS6").

5.2 pKs OF SELECTED ORGANIC ACIDS

The following tables list the pKs of selected organic acids in alphabetical order and in ascending order of pK. Organic acids elute in approximately the order of ascending pK but additional hydrogen bonding and adsorption variables modify the elution order slightly so that the elution order of the acids is not strictly in order of ascending pK. The tables plus the example applications are designed to give the chromatographer a simple method for estimated the ability of the ICE-AS6 to separate various combinations of organic acids.

Table 3
Organic Acids with pK's In Order of Increasing pK_1

Compound	pK_1	pK_2	pK_3
Aconitic (cis-propene-1,2,3-tricarboxylic)			
2-Hydroxyisobutyric	3.72		
Terephthalic			
Squaric (3,4-dihydroxy-3-cyclobutene-1,2-dione	0.40	3.10	
Trichloroacetic	0.66		
Mellitic (benzenehexacarboxylic)	0.70	2.21	3.52
Dichloroacetic	0.87		
Oxalic (ethanedioic)	1.04	3.82	
Nitroacetic	1.46		
Maleic (cis-butenedioic)	1.75	5.83	
Ketoglutaric (2-oxopentanedioic)	1.85	4.44	
Orotic (uracil-6-carboxylic)	1.96	9.34	
Citraconic (cis-methylbutenedioic)	2.20	5.60	
Pyruvic (2-oxopropanoic)	2.26		
Trimellitic (benzene-1,2,4-tricarboxylic)	2.40	3.71	5.01
Fluoroacetic	2.59		
Mesaconic (trans-methylbutene)	2.61		
Cyanoacetic	2.63		
Malonic (propanedioic)	2.65	5.28	
Chloroacetic	2.68		
Gentistic (5-hydroxysalicylic)	2.70		
Dithiotartaric (2,3-dimercaptobutanedioic)	2.71	3.48	8.89
Bromoacetic	2.72		
Phthalic (benzene-1,2-dicarboxylic)	2.75	4.93	
Diglycolic (oxydiacetic)	2.79	3.93	
Salicylic (2-hydroxybenzoic)	2.81	13.40	
Tartaric (d-2,3-dihydroxybutanedioic)	2.82	3.95	
Fumaric (trans-butenedioic)	2.85	4.10	
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	2.87	4.35	5.69
Iodoacetic	2.98		
Isocitrate (dl-1-hydroxypropane-1,2,3-tricarboxylic)	3.02	4.28	5.75
Mucic	3.08	3.63	
Mandelic (l-phenylhydroxyacetic)	3.19		
Galacturonic	3.23	11.42	
Malic (l-hydroxybutanedioic)	3.24	4.71	
Thiomalic (dl-mercaptobutanedioic)	3.30	4.60	10.38
Quinic (1,3,4,5-tetrahydroxycyclohexanecarboxylic)	3.36		
Thioglycolic (mercaptoacetic)	3.42	10.11	
Thiolactic (dl-2-mercaptopropanoic)	3.48	10.08	
Hippuric (n-benzoylglycine)	3.50		
Glyceric (dl-2,3-dihydroxypropanoic)	3.52		
Formic (methanoic)	3.55		
Glycolic (hydroxyacetic)	3.63		
Lactic (d-2-hydroxypropanoic)	3.66		
Itaconic (methylenebutanedioic)	3.68	5.14	
Benzoic	4.00		
Succinic (butanedioic)	4.00	5.24	
Ascorbic	4.03	11.34	
4-Hydroxybenzoic	4.10	9.96	
Vinylacetic (but-3-enoic)	4.12		
Glutaric (pentanedioic)	4.13	5.03	
Acrylic (propenoic)	4.26		
Adipic (hexanedioic)	4.26	5.03	
Pimelic (heptanedioic)	4.31	5.08	
3-Mercaptopropanoic	4.34	10.84	
Azelaic (nonanedioic)	4.39	5.12	
Anisic (4-methoxybenzoic)	4.48		
Acetic (ethanoic)	4.56		
Isovaleric (3-methylbutanoic)	4.58		
Butanoic	4.63		
Isobutyric (2-methylpropionic)	4.63		
Valeric (pentanoic)	4.64		
Propanoic	4.67		
Crotonic (trans-but-2-enoic)	4.69		
Pivalic (2,2-dimethylpropanoic)	4.83		
Caproic (hexanoic)	4.85		
Octanoic	4.89		
Uric (2,6,8-trihydroxypurine)	5.61		
Guanidine	13.54		

Table 4
Organic Acids with pK's in Alphabetic Order

Compound	pK_1	pK_2	pK_3
Acetic (ethanoic)	4.56		
Aconitic (cis-propene-1,2,3-tricarboxylic)			
Acrylic (propenoic)	4.26		
Adipic (hexanedioic)	4.26	5.03	
Anisic (4-methoxybenzoic)	4.48		
Ascorbic	4.03	11.34	
Azelaic (nonanedioic)	4.39	5.12	
Benzoic	4.00		
Bromoacetic	2.72		
Butanoic	4.63		
Caproic (hexanoic)	4.85		
Chloroacetic	2.68		
Citraconic (cis-methylbutenedioic)	2.20	5.60	
Citric (2-hydroxypropane-1,2,3-tricarboxylic)	2.87	4.35	5.69
Crotonic (trans-but-2-enoic)	4.69		
Cyanoacetic	2.63		
Dichloroacetic	0.87		
Diglycolic (oxydiacetic)	2.79	3.93	
Dithiotartaric (2,3-dimercaptobutanedioic)	2.71	3.48	8.89
Fluoroacetic	2.59		
Formic (methanoic)	3.55		
Fumaric (trans-butenedioic)	2.85	4.10	
Galacturonic	3.23	11.42	
Gentistic (5-hydroxysalicylic)	2.70		
Glutaric (pentanedioic)	4.13	5.03	
Glyceric (dl-2,3-dihydroxypropanoic)	3.52		
Glycolic (hydroxyacetic)	3.63		
Guanidine	13.54		
2-Hydroxyisobutyric	3.72		
4-Hydroxybenzoic	4.10	9.96	
Hippuric (n-benzoylglycine)	3.50		
Iodoacetic	2.98		
Isobutyric (2-methylpropionic)	4.63		
Isocitrate (dl-1-hydroxypropane-1,2,3-tricarboxylic)	3.02	4.28	5.75
Isovaleric (3-methylbutanoic)	4.58		
Itaconic (methylenebutanedioic)	3.68	5.14	
Ketoglutaric (2-oxopentanedioic)	1.85	4.44	
Lactic (d-2-hydroxypropanoic)	3.66		
Maleic (cis-butenedioic)	1.75	5.83	
Malic (l-hydroxybutanedioic)	3.24	4.71	
Malonic (propanedioic)	2.65	5.28	
Mandelic (l-phenylhydroxyacetic)	3.19		
Mellitic (benzenehexacarboxylic)	0.70	2.21	3.52
3-Mercaptopropanoic	4.34	10.84	
Mesaconic (trans-methylbutene)	2.61		
Mucic	3.08	3.63	
Nitroacetic	1.46		
Octanoic	4.89		
Orotic (uracil-6-carboxylic)	1.96	9.34	
Oxalic (ethanedioic)	1.04	3.82	
Phthalic (benzene-1,2-dicarboxylic)	2.75	4.93	
Pimelic (heptanedioic)	4.31	5.08	
Pivalic (2,2-dimethylpropanoic)	4.83		
Propanoic	4.67		
Pyruvic (2-oxopropanoic)	2.26		
Quinic (1,3,4,5-tetrahydroxycyclohexanecarboxylic)	3.36		
Salicylic (2-hydroxybenzoic)	2.81	13.40	
Squaric (3,4-dihydroxy-3-cyclobutene-1,2-dione	0.40	3.10	
Succinic (butanedioic)	4.00	5.24	
Tartaric (d-2,3-dihydroxybutanedioic)	2.82	3.95	
Terephthalic			
Thioglycolic (mercaptoacetic)	3.42	10.11	
Thiolactic (dl-2-mercaptopropanoic)	3.48	10.08	
Thiomalic (dl-mercaptobutanedioic)	3.30	4.60	10.38
Trichloroacetic	0.66		
Trimellitic (benzene-1,2,4-tricarboxylic)	2.40	3.71	5.01
Uric (2,6,8-trihydroxypurine)	5.61		
Valeric (pentanoic)	4.64		
Vinylacetic (but-3-enoic)	4.12		

5.3 PRODUCTION TEST CHROMATOGRAM

5.3.1 DISCUSSION OF METHOD

Isocratic elution of organic anions has been optimized on the IonPac ICE-AS6 Analytical Column. To guarantee that all IonPac ICE-AS6 Analytical Columns meet high quality and reproducible performance specification standards, all columns undergo the following production control test.

The large dip in the baseline which occurs at approximately 5 minutes is the total exclusion volume of the column. This is the volume of liquid in the column which is external to the resin beads. The total permeation or void volume of the column, the combined resin bead internal and external liquid volume, creates a baseline disturbance at approximately 13 minutes. This baseline disturbance, however, can only be seen at sensitivities higher than 1 µS full scale.

5.3.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi
Storage Solution:	Eluent, 0.4 mM Heptafluorobutyric acid

5.3.3 EXAMPLE CHROMATOGRAM

	Acid	pK's	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 5.28	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0

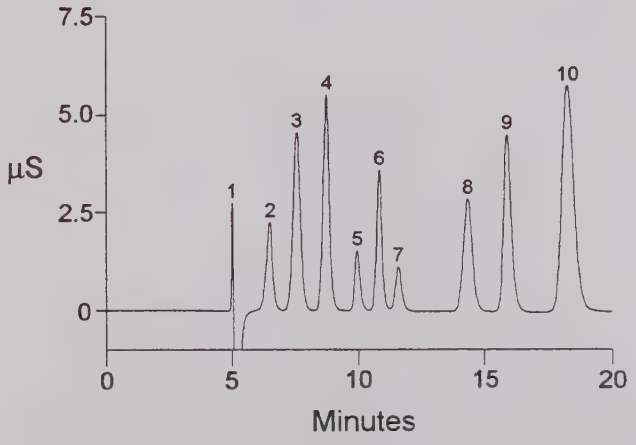


Figure 3

IonPac ICE-AS6 Production Test Chromatogram

5.4 FULL IONPAC ICE-AS6 ORGANIC ACID CHROMATOGRAM

5.4.1 DISCUSSION OF METHOD

Fumarate, propionate and glutarate will also elute using the Production Test Chromatogram conditions if the run is allowed to proceed to 40 minutes.

5.4.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi
Storage Solution:	Eluent, 0.4 mM Heptafluorobutyric acid

5.4.3 EXAMPLE CHROMATOGRAM

	Acid	pK's	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 5.28	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0
11.	Fumaric	2.85, 4.10	35.0
12.	Propionic	4.67	50.0
13.	Glutaric	4.13,5.03	40.0

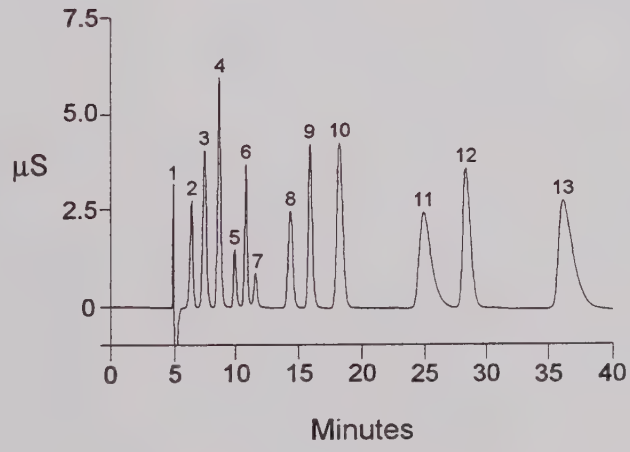


Figure 4

Full IonPac ICE-AS6 Organic Acid Chromatogram

5.5 COMPARISON OF DIFFERENT ACIDS USED ON THE IONPAC ICE-AS6

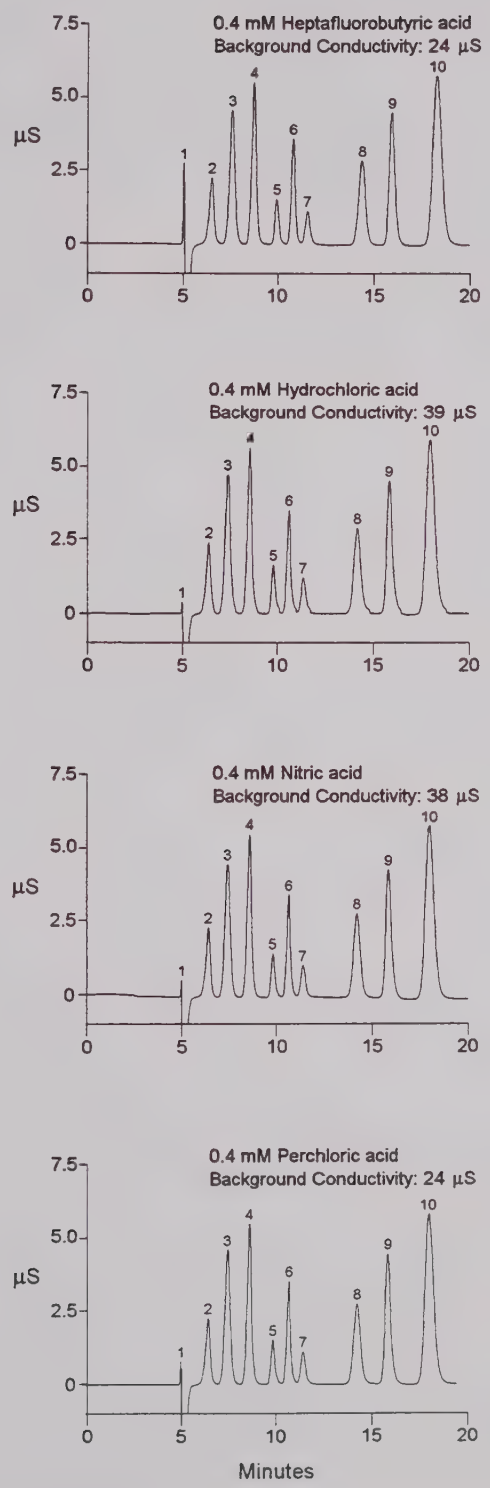
5.5.1 DISCUSSION OF METHOD

The following examples demonstrate that at constant concentration, several different acids are observed to yield chromatograms of identical selectivity, sensitivity and runtime.

5.5.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	See Chromatogram (0.4 mM each)
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.5.3 EXAMPLE CHROMATOGRAMS



	Acid	pK's	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 5.28	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0

Figure 5

Comparison of Different Acids used with the IonPac ICE-AS6

5.6 RETENTION TIMES VERSUS ELUENT ACID CONCENTRATION

5.6.1 DISCUSSION OF METHOD

This example compares predicted separations obtained from a plot of column selectivity versus eluent strength, to the actual chromatographic separations. For the first estimate of the best separation based on retention times only, the selectivity chart is used. The actual resolution of separation though is also dependent on the efficiency of the column.

Note that below 0.2 mM heptafluorobutyric acid, the retention times of all of the analytes fall off very quickly. On close examination, there is a minimum eluent concentration required to maintain peak symmetry which in this case is also approximately 0.2 mM heptafluorobutyric acid.

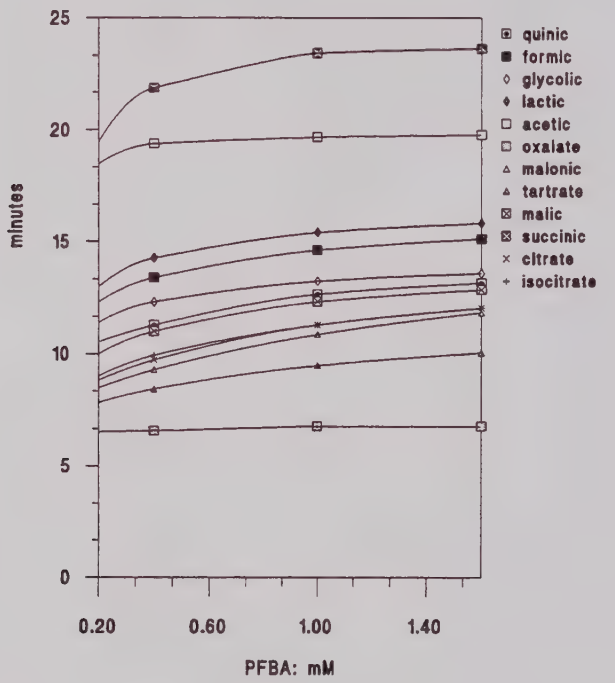


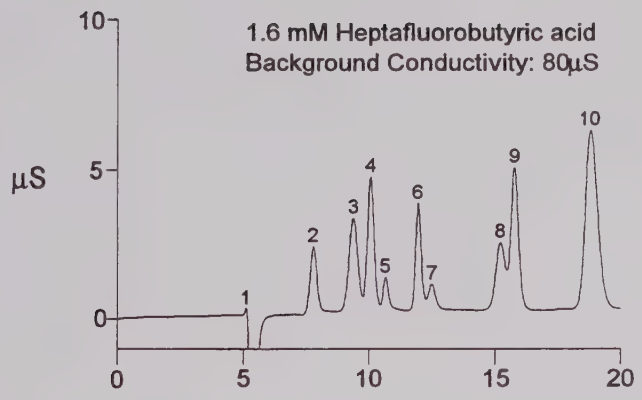
Figure 5

Plot of Retention Time versus Eluent Concentration

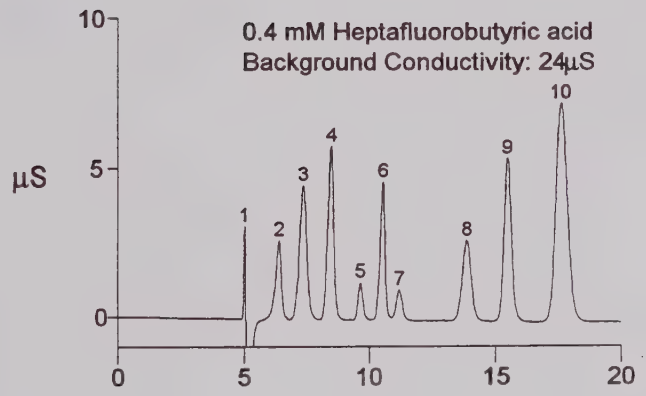
5.6.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	See Chromatogram
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	See Chromatogram
Expected System Operating Back pressure:	<440 psi

5.6.3 EXAMPLE CHROMATOGRAMS



	Acid	pK's	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 5.28	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	40.0



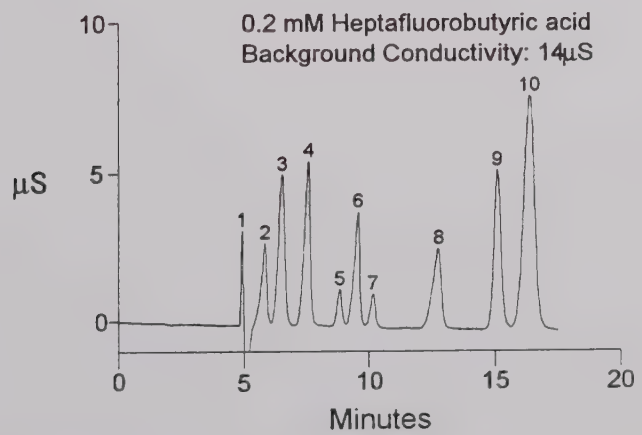


Figure 7

IonPac ICE-AS6 Retention Time versus Acid Concentration

5.7 THE ADDITION OF SOLVENT TO THE ELUENT

5.7.1 DISCUSSION OF METHOD

The following example demonstrates the separation of C1 to C6 organic acids with the addition of 20% acetonitrile to the standard 0.4 mM heptafluorobutyric acid eluent.

5.7.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid/20% ACN
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.7.3 EXAMPLE CHROMATOGRAM

	Acid	pK's	mg/L
1.	Formic	3.55	
2.	Acetic	4.56	
3.	Propionic	4.83	
4.	Butyric	4.00	
5.	Valeric	4.64	
6.	Caproic	4.85	

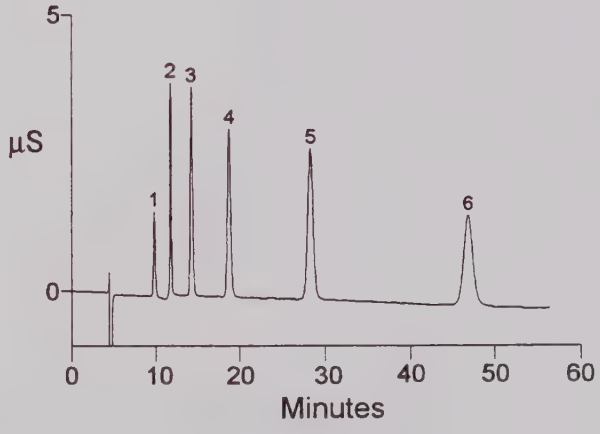


Figure 8

IonPac ICE-AS6 Chromatography with Solvent Eluent

5.8 ANALYSIS OF A COFFEE SAMPLE

5.8.1 DISCUSSION OF METHOD

The following analysis of coffee demonstrates one of the most complex samples. Because of the complexity of the sample, concentrated coffee samples were used for the fouling studies.

5.8.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.8.3 EXAMPLE CHROMATOGRAM

	Acid	pK's
1.	Inorganics	
2.	Inorganics	
3.	Maleic	3.24,4.71
4.	Malonic	2.65,5.28
5.	Citric	2.87, 4.35, 5.69
6.	Malic	3.24, 5.28
7.	Quinic	3.36
8.	Glycolic	3.63
9.	Formic	3.55
10.	Lactic	3.66
11.	Unknown	
12.	Acetic	4.56
13.	Unknown	
14.	Shikimic	
15.	Fumaric	3.55
16.	Propionic	4.83

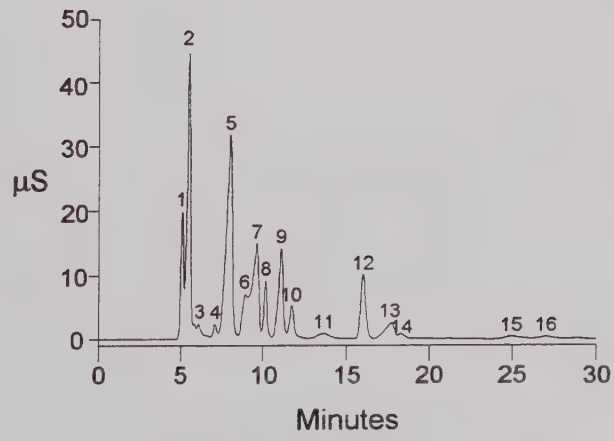


Figure 9

IonPac ICE-AS6 Analysis of Coffee

5.9 FOULING RESISTANCE OF IONPAC ICE-AS6 COLUMNS

5.9.1 DISCUSSION OF METHOD

The following fouling runs used a 10X concentrated instant coffee solution. After 100 injections of the column with the concentrate, the standard 10 organic acids were analyzed to demonstrate the degree of column degradation. Note that after the 100 injections of the 10X coffee concentrate the pressure had increased from 420 to 510 psi.

5.9.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	See Chromatogram

5.9.3 EXAMPLE CHROMATOGRAMS

	Acid	pK's	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 5.28	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	40.0

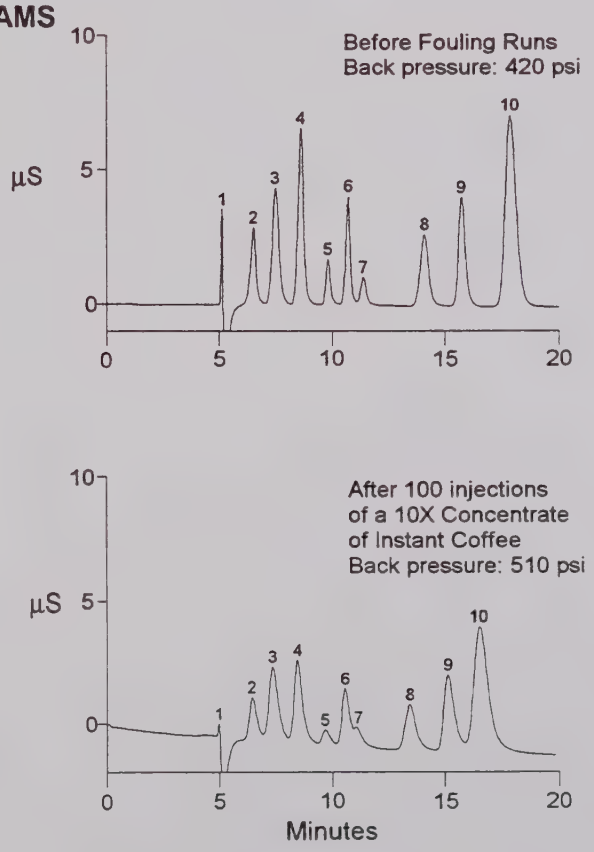


Figure 10

Fouling Resistance of IonPac ICE-AS6 Columns

5.10 ANALYSIS OF MILK

5.10.1 DISCUSSION OF METHOD

The following example demonstrates the analysis of milk on the IonPac ICE-AS6.

5.10.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.10.3 EXAMPLE CHROMATOGRAM

	Acid	pK's
1.	Inorganics	
2.	Inorganics	
3.	Citric	2.87, 4.35, 5.69
4.	Formic	3.55

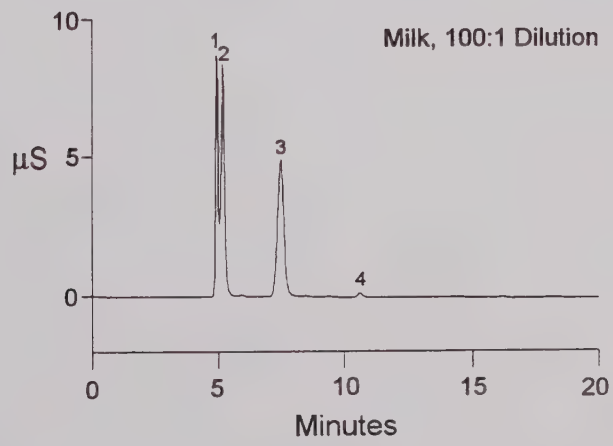


Figure 11

IonPac ICE-AS6 Analysis of Milk

5.11 ANALYSIS OF WINE SAMPLES

5.11.1 DISCUSSION OF METHOD

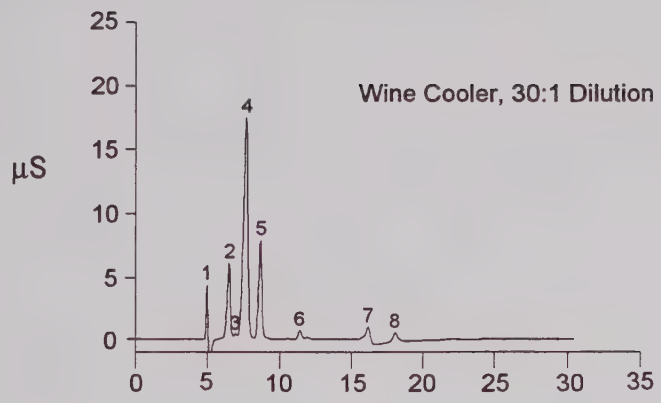
The following samples demonstrate the analysis of different wine samples on the IonPac ICE-AS6.

5.11.2 GENERAL OPERATING CONDITIONS

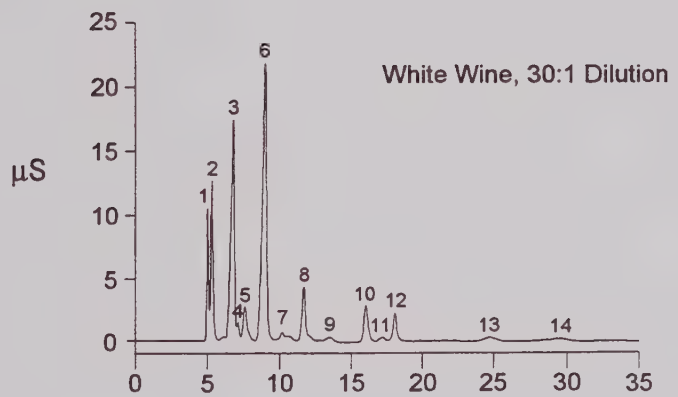
Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.11.3 EXAMPLE CHROMATOGRAMS

	Acid	pK's
1.	Inorganics	
2.	Tartaric	2.82, 3.95
3.	Malonic	3.19
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 5.28
6.	Lactic	3.66
7.	Carbonic	
8.	Succinic	4.00, 5.24



	Acid	pK's
1.	Inorganics	
2.	Inorganics	
3.	Tartaric	2.82, 3.95
4.	Galaturonic	3.23,11.42
5.	Citric	2.87, 4.35, 5.69
6.	Malic	3.24, 5.28
7.	Glycolic	3.63
8.	Lactic	3.66
9.	Unknown	
10.	Acetic	4.56
11.	Unknown	
12.	Succinic	4.00, 5.24
13.	Fumaric	3.55
14.	Propionic	4.83



	Acid	pK's
1.	Inorganics	
2.	Inorganics	
3.	Unknown	
4.	Tartaric	2.82, 3.95
5.	Galaturonic	3.23,11.42
6.	Citric	2.87, 4.35, 5.69
7.	Malic	3.24, 5.28
8.	Unknown	
9.	Glycolic	3.63
10.	Lactic	3.66
11.	Unknown	
12.	Acetic	4.56
13.	Succinic	4.00, 5.24

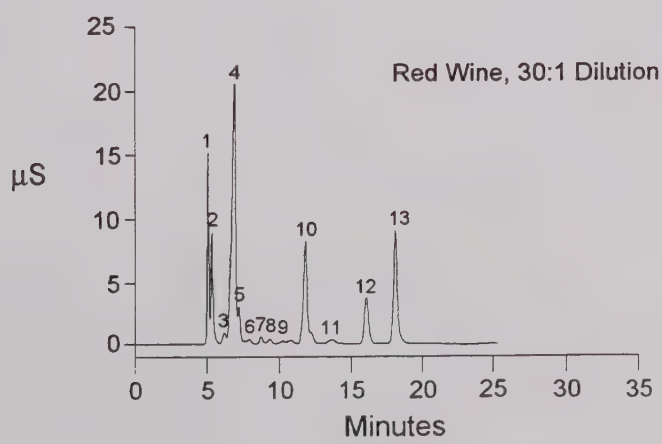


Figure 12

IonPac ICE-AS6 Analysis of Wine Samples

5.12 ANALYSIS OF JACK DANIELS LYNCHBURGH LEMONADE

5.12.1 DISCUSSION OF METHOD

The following example demonstrates the analysis of an alcoholic beverage on the IonPac ICE-AS6.

5.12.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.12.3 EXAMPLE CHROMATOGRAM

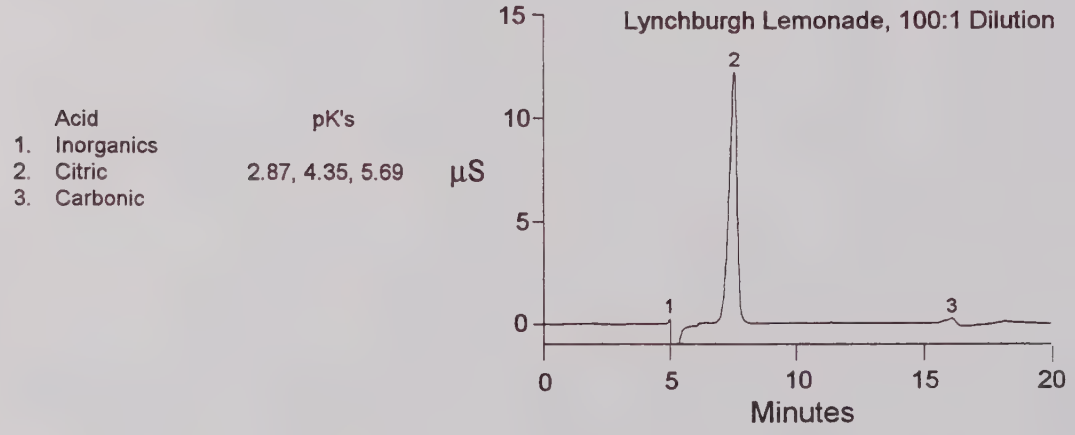


Figure 13

IonPac ICE-AS6 Analysis of Jack Daniels Lynchburgh Lemonade

5.13 ANALYSIS OF BEER SAMPLES

5.13.1 DISCUSSION OF METHOD

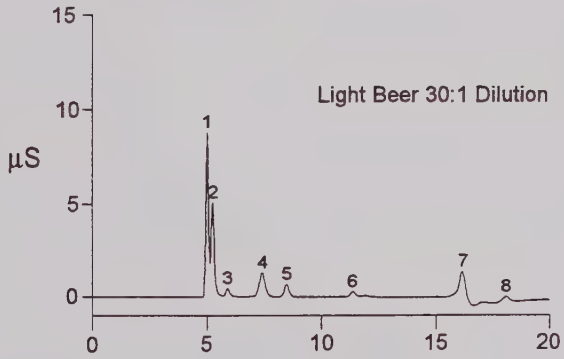
The following examples demonstrate the analysis of beer samples on the IonPac ICE-AS6.

5.13.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.13.3 EXAMPLE CHROMATOGRAMS

	Acid	pK's
1.	Inorganics	
2.	Inorganics	
3.	Maleic	3.24,4.71
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 5.28
6.	Lactic	3.66
7.	Carbonic	
8.	Succinic	4.00,5.24



	Acid	pK's
1.	Inorganics	
2.	Inorganics	
3.	Maleic	3.24,4.71
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 5.28
6.	Formic	3.55
7.	Lactic	3.66
8.	Carbonic	
9.	Succinic	4.00,5.24

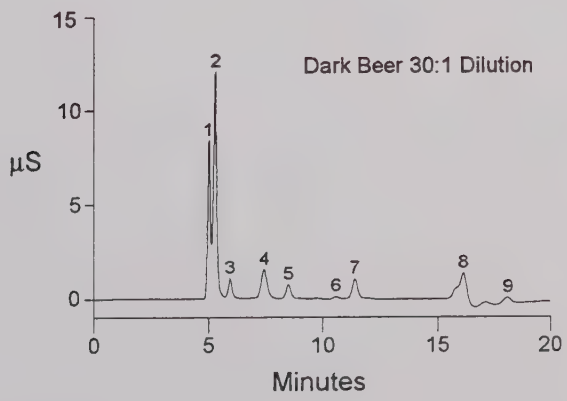


Figure 14

IonPac ICE-AS6 Analysis of Beer Samples

5.14 ANALYSIS OF FRUIT JUICE SAMPLES

5.14.1 DISCUSSION OF METHOD

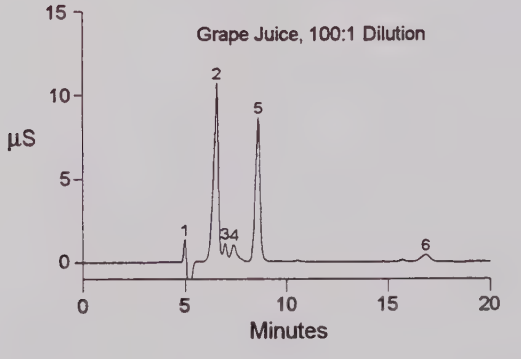
The following examples demonstrate the analysis of various fruit juices on the IonPac ICE-AS6.

5.14.2 GENERAL OPERATING CONDITIONS

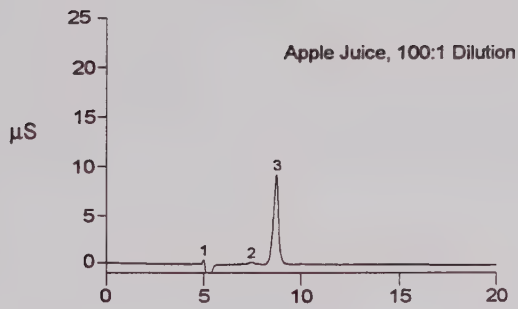
Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.14.3 EXAMPLE CHROMATOGRAMS

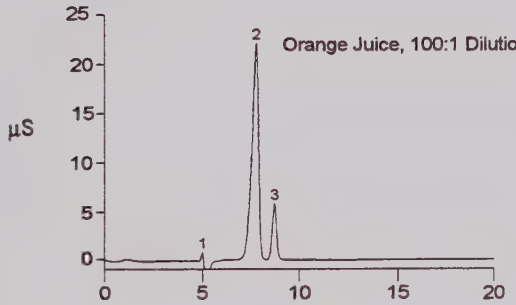
	Acid	pK's
1.	Inorganics	
2.	Tartaric	2.82,3.95
3.	Galacturonic	3.23,11.42
4.	Citric	2.87, 4.35, 5.69
5.	Malic	3.24, 5.28
6.	Unknown	



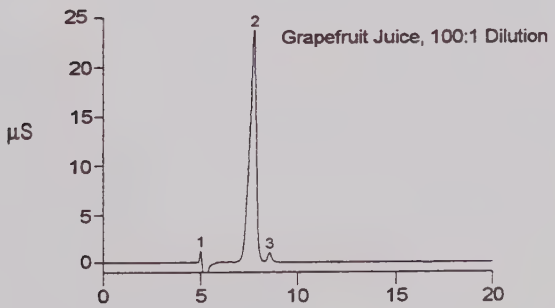
	Acid	pK's
1.	Oxalic	1.04,3.82
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28



	Acid	pK's
1.	Oxalic	1.04,3.82
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28



	Acid	pK's
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28



	Acid	pK's
1.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28

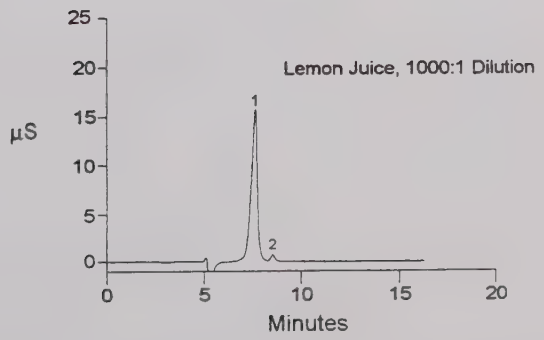


Figure 15

IonPac ICE-AS6 Analysis of Fruit Juices

5.15 ANALYSIS OF TOMATO PRODUCT SAMPLES

5.15.1 DISCUSSION OF METHOD

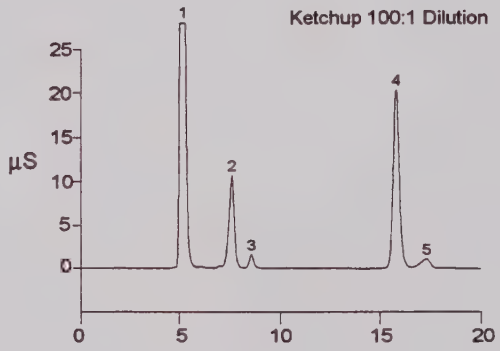
The following examples demonstrate the analysis of tomato products on the IonPac ICE-AS6.

5.15.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.15.3 EXAMPLE CHROMATOGRAMS

	Acid	pK's
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28
4.	Acetate	4.56
5.	Unknown	



	Acid	pK's
1.	Inorganics	
2.	Malonic	2.65,5.28
3.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28
4.	Formic	3.55
5.	Unknown	

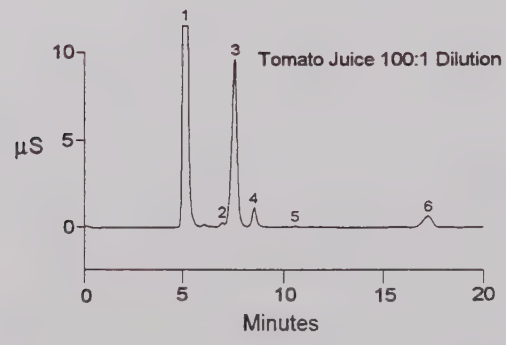


Figure 16

IonPac ICE-AS6 Analysis of Tomato Products

5.16 ANALYSIS OF MUSTARD

5.16.1 DISCUSSION OF METHOD

The following examples demonstrate the analysis of mustard on the IonPac ICE-AS6.

5.16.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.16.3 EXAMPLE CHROMATOGRAM

	Acid	pK's
1.	Inorganics	
2.	Citric	2.87, 4.35, 5.69
3.	Malic	3.24, 5.28
4.	Acetic	4.56

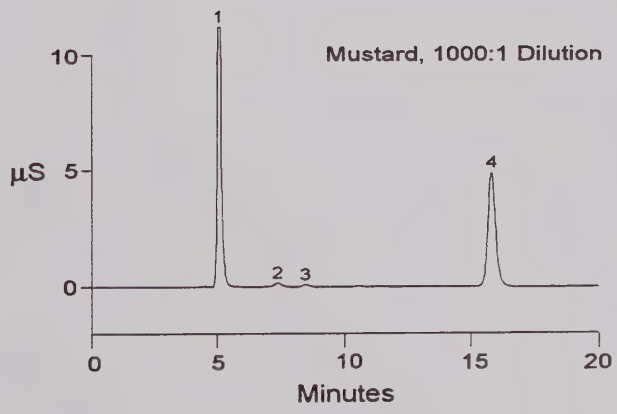


Figure 17

IonPac ICE-AS6 Analysis of Mustard

5.17 ANALYSIS OF BORIC ACID AND CARBONIC ACID

5.17.1 DISCUSSION OF METHOD

The following examples demonstrate the analysis of a sample containing boric acid and carbonic acid on the IonPac ICE-AS6.

5.17.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.17.3 EXAMPLE CHROMATOGRAM

	Acid	pKa	mg/L
1.	Galactronic	3.23,11.42	30.0
2.	Threonic		15.0
3.	Quinic	3.63	20.0
4.	Boric		5.0
5.	Chloroacetic	2.68	10.0
6.	Tricarboxylic		5.0
7.	Carbonic		50.0
8.	Shikimik		50.0
9.	Itaconic	3.68,5.14	15.0
10.	Acrylic	4.26	30.0

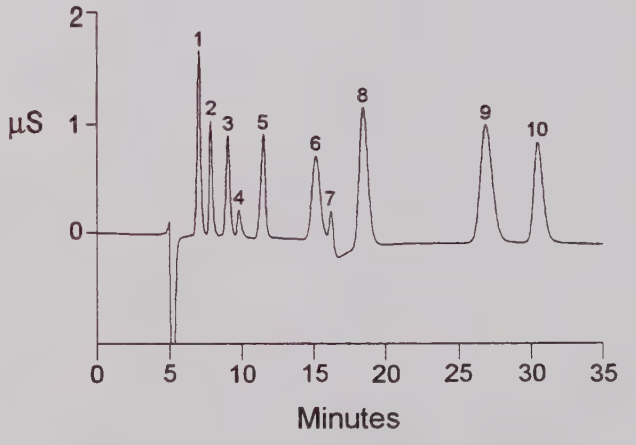


Figure 18

IonPac ICE-AS6 Analysis of Boric Acid and Carbonic Acid

5.18 ANALYSIS OF 0.25% SULFURIC ACID

5.18.1 DISCUSSION OF METHOD

The following example demonstrates the analysis of organic acids in 0.25% sulfuric acid on the IonPac ICE-AS6.

5.18.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL, 0.25% Sulfuric Acid
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	1.0 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	73-75 µS
Expected System Operating Back pressure:	<500 psi

5.18.3 EXAMPLE CHROMATOGRAM

	Analyte	mg/L
1.	Citrate	0.5
2.	Malic	1.0
3.	Glycolic	1.0
4.	Lactic	1.0
5.	Acetic	1.0
6.	Succinic	1.0

where 1 mg/L = 1 ppm

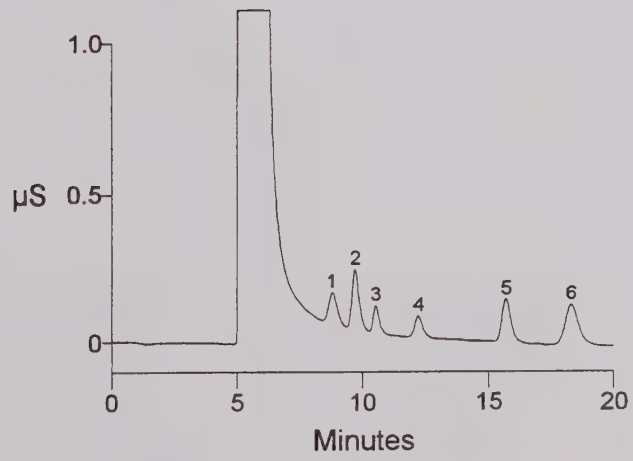


Figure 19

IonPac ICE-AS6 Analysis of 0.25% Sulfuric Acid

5.19 ANALYSIS OF 0.50% SODIUM CHLORIDE

5.19.1 DISCUSSION OF METHOD

The following example demonstrates the analysis of organic acids in 0.50% sodium chloride on the IonPac ICE-AS6.

5.19.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL, 0.5% Sodium Chloride
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	1.0 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	73-75 µS
Expected System Operating Back pressure:	<500 psi

5.19.3 EXAMPLE CHROMATOGRAM

	Analyte	mg/L
1.	Tartrate	1.0
2.	Citrate	0.5
3.	Malic	1.0
4.	Glycolic	1.0
5.	Lactic	1.0
6.	Acetic	1.0
7.	Succinic	1.0

where 1 mg/L = 1 ppm

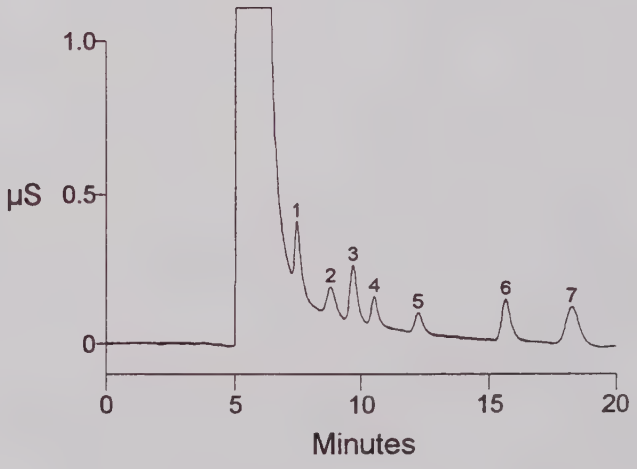


Figure 20

IonPac ICE-AS6 Analysis of 0.25% Sodium Chloride

5.20 COMPARISON OF UV VERSUS SUPPRESSED CONDUCTIVITY DETECTION

5.20.1 DISCUSSION OF METHOD

The following example demonstrates the comparison of UV detection and suppressed conductivity detection of organic acids on the IonPac ICE-AS6.

5.20.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 µL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 µS
Expected System Operating Back pressure:	<500 psi

5.20.3 EXAMPLE CHROMATOGRAMS

	Acid	pK's	mg/L
1.	Oxalic	1.04, 3.82	5.0
2.	Tartaric	2.82, 3.95	10.0
3.	Citric	2.87, 4.35, 5.69	15.0
4.	Malic	3.24, 5.28	20.0
5.	Glycolic	3.63	10.0
6.	Formic	3.55	10.0
7.	Lactic	3.66	10.0
8.	HIBA	3.72	30.0
9.	Acetic	4.56	25.0
10.	Succinic	4.00, 5.24	25.0
11.	Fumaric	2.85, 4.10	35.0
12.	Propionic	4.67	50.0
13.	Glutaric	4.13,5.03	40.0

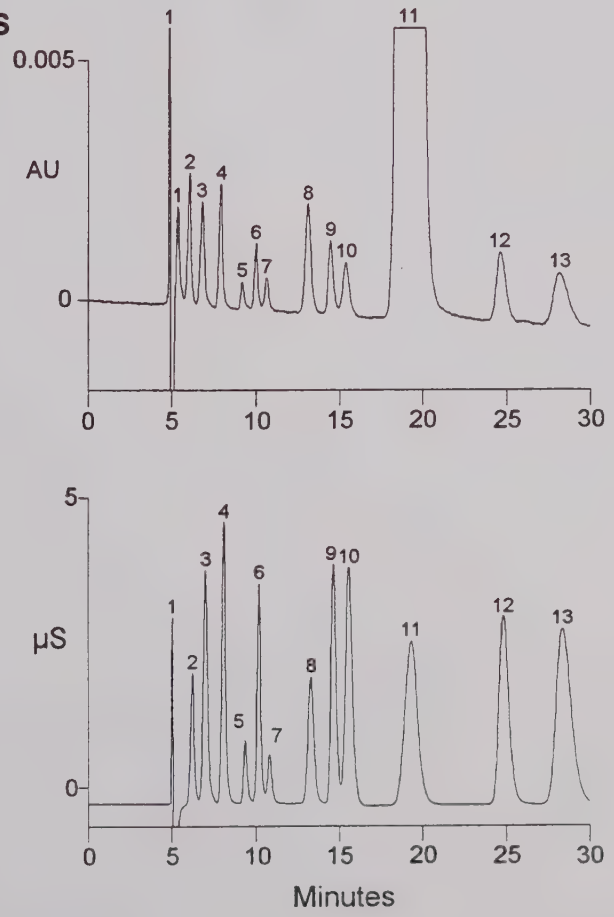


Figure 21

Comparison of UV detection and Suppressed Conductivity Detection

5.21 DETECTION OF ALCOHOLS USING PULSE AMPEROMETRIC DETECTION

5.21.1 DISCUSSION OF METHOD

The top chromatogram illustrates pulsed amperometric detection of alcohols in which the eluent and the sample are carefully degassed.*

The middle chromatogram illustrates the appearance of the total permeation volume baseline dip when the eluent and sample are not thoroughly degassed.

The bottom chromatogram illustrates the repositioning of the total permeation volume baseline dip with the addition of an IonPac NG1 in front of the IonPac ICE-AS6. The added permeation volume of the IonPac NG1 provides improved resolution of methanol and propylene glycol.

* A woven reaction coil has been used to equilibrate oxygen levels with similar results.

5.21.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume: 100 µL (sample made up in eluent and degassed)
Analytical Column: IonPac ICE-AS6 Analytical Column
Eluent: 75 mM $HClO_4$ (made fresh daily and sonicated under vacuum for 15 minutes to degas)
Eluent Flow Rate: 1.5 mL/min
Detection: PED with 3 mm Platinum Electrode

Waveform		Integration	
Time (sec)	Poten (V)	Begin (sec)	End (sec)
0.00	0.30	0.01	0.21
0.30	0.30		
0.31	1.40		
0.51	1.40		
0.52	-1.40		
0.92	-1.40		

5.21.3 EXAMPLE CHROMATOGRAMS

	Analyte	mg/L
1.	Sorbitol	1000
2.	Erythritol	1000
3.	Glycerol	1000
4.	Ethylene Glycol	1000
5.	Methanol	1000
6.	Propylene Glycol	1000
7.	Ethanol	1000
8.	2-Propanol	1000
9.	1-Propanol	1000

where 1 mg/L = 1 ppm

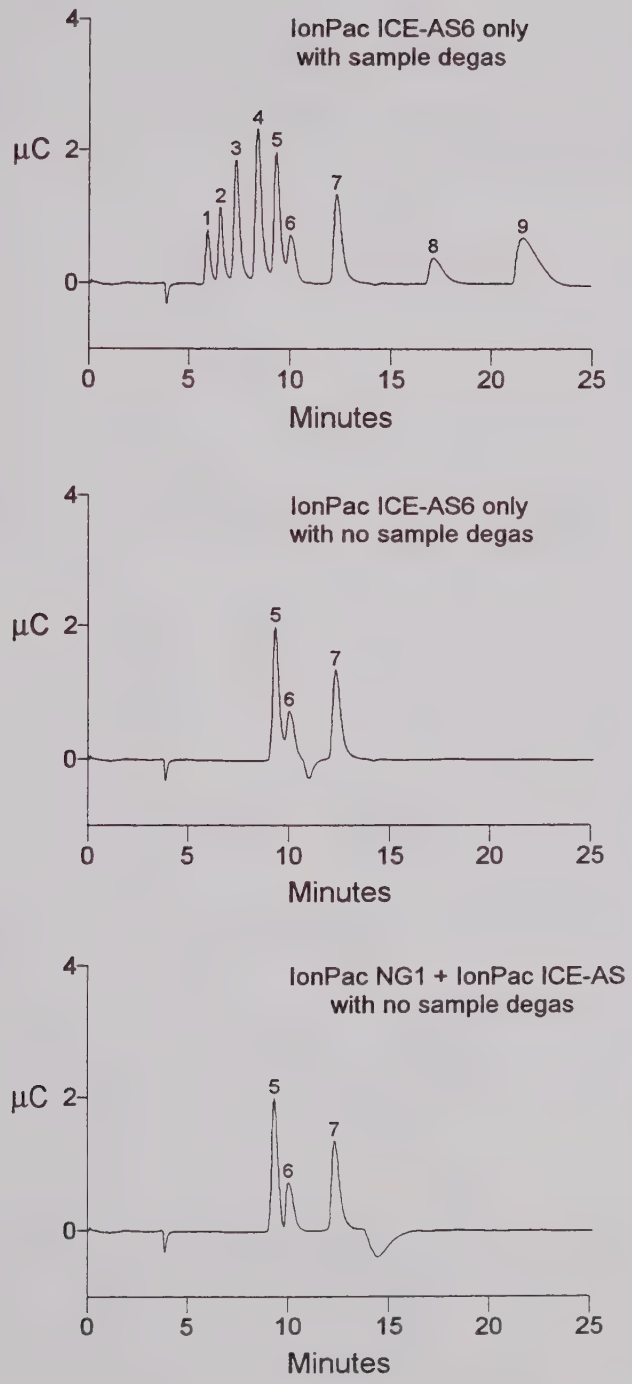


Figure 22

Detection of Alcohols Using Pulsed Amperometric Detection

5.22 ANALYSIS OF SELECTED INORGANIC IONS

5.22.1 DISCUSSION OF METHOD

The following examples demonstrate the analysis of selected inorganic ions on the IonPac ICE-AS6.

5.22.2 GENERAL OPERATING CONDITIONS

Sample Loop Volume:	50 mL
Analytical Column:	IonPac ICE-AS6 Analytical Column
Eluent:	0.4 mM Heptafluorobutyric acid
Eluent Flow Rate:	1.0 mL/min
Suppressor:	Anion-ICE MicroMembrane Suppressor
Regenerant;	5 mN Tetrabutylammonium hydroxide
Regenerant Flow :	5 mL/min
Expected Background Conductivity:	23-25 mS
Expected System Operating Back pressure:	<500 psi

5.22.3 EXAMPLE CHROMATOGRAM

Ion
1. Nitrate/Chloride/Sulfate
2. Phosphate
3. Fluoride (HF_2^-)
(Total concentration: ~ 300 mg/L)

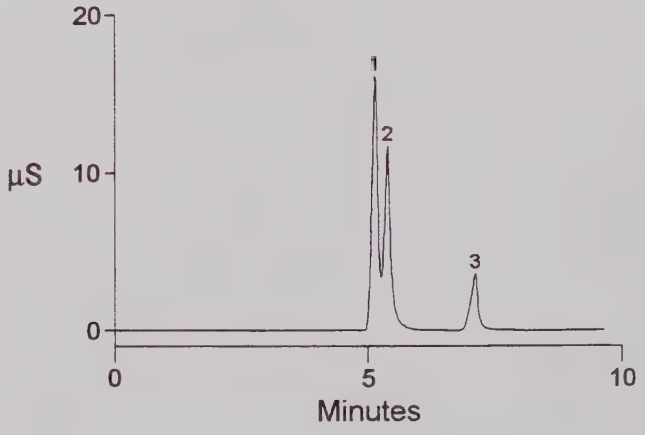


Figure 23

IonPac ICE-AS6 Analysis of Selected Inorganic Anions

SECTION 6 - TROUBLESHOOTING GUIDE

The purpose of the Troubleshooting Guide is to help you solve operating problems that may arise while using the IonPac ICE-AS6 column. For more information on problems that originate with the Ion Chromatograph (IC) or the Anion-ICE MicroMembrane Suppressor (AMMS-ICE), refer to the Troubleshooting Guide in the appropriate operator's manual. If you cannot solve the problem on your own, contact the DIONEX Regional Office nearest you (see Section 10, "DIONEX Regional Offices").

6.1 HIGH BACK PRESSURE

6.1.1 FINDING THE SOURCE OF HIGH SYSTEM PRESSURE

Total system pressure when using the IonPac ICE-AS6 Analytical Column at 1.0 mL/min should be less than 550 psi when using the eluent used to generate the test chromatogram. Refer to Section 4.1.2, "Solvents", to see how solvent concentration can affect the column operating pressure. If the system pressure is higher than 600 psi, it is advisable to find out what is causing the high system pressure.

The system should be used with a High-Pressure In-Line Filter (P/N 035331) for eluents. The filter should be positioned between the Advanced Gradient Pump (AGP-1) pressure transducer and the injection valve. Since the liquid lines on the AGP-1 have 10-32 ferrule/bolt fittings and the High-Pressure In-Line Filter has one male 1/4-28 fitting and one female 1/4-28 port, it is necessary to install two adaptor assemblies on the filter. On the end of the filter with the 1/4-28 male fitting, place a 1/4-28 to 10-32 union (P/N 042806). On the end of the filter with the 1/4-28 female port place a 1/4-28 male to 10-32 female port adaptor assembly (P/N 043291). Make sure you have a High-Pressure In-Line Filter in place and that it is not contaminated.

A. Make sure that the pump is set to the correct eluent flow rate. Higher than recommended eluent flow rates will cause higher pressure. Measure the pump flow rate if necessary with a stop watch and graduated cylinder.

B. Find out what part of the system is causing the high pressure. It could be a piece of tubing that has plugged or whose walls are collapsed, an injection valve with a plugged port, a column with particulates plugging the bed support, a plugged High-Pressure In-Line Filter, the AMMS-ICE, or the detector cell.

   To find out which part of the chromatographic system is causing the problem, disconnect the pump eluent line from the injection valve and turn the pump on. Watch the pressure; it should not exceed 50 psi. Continue adding the system's components (injection valve, column(s), AMMS-ICE and the detector) one by one, while watching the system pressure. The pressure should increase up to a maximum of 550 psi at a flow rate of 2.0 mL/min when the column(s) are connected. The AMMS-ICE may add up to 130 psi. No other components should add more than 100 psi of pressure. Refer to the appropriate manual for cleanup or replacement of the problem component.

6.1.2 REPLACING COLUMN BED SUPPORT ASSEMBLIES

If the column inlet bed support is determined to be the cause of the high back pressure, it should be replaced. To change the inlet bed support assembly, refer to the following instructions, using one of the two spare inlet bed support assemblies included in the Ship Kit.

A. Disconnect the column from the system.

B. Using two open end wrenches, carefully unscrew the inlet (top) column fitting. If the resin appears soupy, carefully remove excess moisture by touching a paper towel to the edge of the resin bed. Allow water (not resin) to soak into the paper towel.

C. Turn the end fitting over and tap it against a benchtop or other hard, flat surface to remove the bed support and seal assembly. If the bed support must be pried out of the end fitting, use a sharp pointed object such as a pair of tweezers, but be careful that you **DO NOT SCRATCH THE WALLS OF THE END FITTING.** Discard the old bed support assembly.

D. Place a new bed support assembly into the end fitting. Make sure that the end of the column tube is clean and free of any particulate matter so that it will properly seal against the bed support assembly. Use the end of the column to carefully start the bed support assembly into the end fitting.

	IonPac ICE-AS6 Columns (P/N)
Analytical Column	046023
Bed Support Assembly	048238
Zitex® Bed Support	048297
End Fitting	048298

CAUTION

If the column tube end is not clean when inserted into the end fitting, particulate matter may obstruct a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or the end fitting. Carefully wipe the sealing surfaces clean before assembling

E. Screw the end fitting back onto the column. Tighten it fingertight, then an additional 1/4 turn (25 in x lb). Tighten further only if leaks are observed.

F. Reconnect the column to the system and resume operation.

NOTE

DO NOT attempt to remove the outlet column fitting as the resin will extrude out of the column and ruin it.

6.2 HIGH BACKGROUND OR NOISE

In a properly working system, the background conductivity levels expected for several eluent systems are shown below:

ELUENT	EXPECTED BACKGROUND CONDUCTIVITY
0.2 mM heptafluorobutyric acid	13 - 15 µS
0.4 mM heptafluorobutyric acid	23 - 25 µS
1.6 mM heptafluorobutyric acid	78 - 82 µS

6.2.1 PREPARATION OF ELUENTS

A. Make sure that the eluents and the regenerant are made correctly.

B. Make sure that the eluents are made from chemicals with the recommended purity.

C. Make sure that the deionized water used to prepare the reagents has a specific resistance of 17.8 megohm-cm or greater.

6.2.3 A CONTAMINATED GUARD OR ANALYTICAL COLUMN

Remove the IonPac ICE-AS6 Analytical Column from the system. If the background conductivity decreases, then the column is the cause of the high background conductivity, clean the column as instructed in Section 7.4, "Column Cleanup".

6.2.4 CONTAMINATED HARDWARE

To eliminate the hardware as the source of the high background conductivity, bypass the AMMS-ICE and pump deionized water with a specific resistance of 17.8 megohm-cm or greater through the system. The background conductivity should be less than 2 ∝S. If it is not, check the detector/conductivity cell calibration by injecting deionized water directly into it. See the appropriate manual for details.

6.2.5 A CONTAMINATED ANION MICROMEMBRANE SUPPRESSOR (AMMS-ICE)

If the above items have been checked and the problem persists, the AMMS-ICE is probably causing the problem.

A. Check the regenerant flow rate at the REGEN OUT port of the AMMS-ICE. For the example isocratic applications, this flow rate should be 3 - 5 mL/min.

B. Check the eluent flow rate. For most applications, the eluent flow rate should be 0.8 mL/min. Refer to the Anion-ICE MicroMembrane Suppressor Installation Instructions and Troubleshooting Guide (Document No. 032661) to ensure that the eluent is within suppressible limits.

C. Prepare fresh regenerant solution. If the background conductivity is high after preparing fresh regenerant, you probably need to clean or replace your AMMS-ICE. Refer to the "Anion MicroMembrane Suppressor Installation Instructions and Troubleshooting Guide" (Document No. 032661) for assistance.

6.3 POOR PEAK RESOLUTION

Poor peak resolution can be due any or all of the following factors.

6.3.1 LOSS OF COLUMN EFFICIENCY

A. Check to see if headspace has developed in the analytical column (e.g., due to improper use of the column such as submitting it to high pressures). Remove the column's top end fitting (see Section 6.1.2, "Replacing Column Bed Support Assemblies"). If the resin does not fill the column body all the way to the top, it means that the resin bed has collapsed, creating a headspace. 1 - 2 mm of headspace is the maximum allowable before the column demonstrates significant losses of efficiency. If more than 2 mm of headspace is observed, the column must be replaced.

B. Extra-column system effects can result in sample band dispersion, decreasing peak efficiencies. Make sure you are using PEEK tubing with an ID of no greater than 0.010" to make all eluent liquid line connections between the injection valve and the detector cell inlet on 4mm systems. Check for leaks.

6.3.2 POOR RESOLUTION DUE TO SHORTENED RETENTION TIMES

Even with adequate system and column efficiency, resolution of peaks will be compromised if analytes elute too fast.

A. Check to see if the eluent flow rate is different than the flow rate specified by the analytical protocol. Measure the eluent flow rate after the column using a stopwatch and graduated cylinder. Wait at least 5 minutes before making the measurement to allow time for the pump pressure feedback to engage.

B. Check to see if the eluent compositions and concentrations are correct.

   For isocratic analysis, an eluent that is too strong will cause the peaks to elute later. Prepare fresh eluent. If you are using a gradient pump to proportion the final eluent from concentrated eluents in two or three different eluent reservoirs, the composition of the final eluent may not be accurate enough for the application. Use one reservoir containing the correct eluent composition to see if this is the problem. This may be a problem when one of the proportioned eluents is less than 5%.

C. Column contamination can lead to a loss of column efficiency. Cationic contamination nonionic contamination or metal ions might be concentrating on the column. Refer to Section 7.4, "Column Cleanup", for recommended column cleanup procedures.

   Possible sources of column contamination are impurities in chemicals, in the deionized water or from the sample matrix being used. Be especially careful to make sure that the recommended chemicals are used. The deionized water should have a specific resistance of at least 17.8 megohm-cm or better.

D. If run times are reduced to the point that resolution is lost, clean the column (see Section 7.4, "Column Cleanup").

After cleaning the column, reinstall it in the system and let it equilibrate with eluent for about 30 minutes. The column is equilibrated when consecutive injections of the standard give reproducible retention times. The original column capacity should be restored by this treatment, since the contaminants should be eluted from the column. If you need assistance in solving resolution problems, contact the DIONEX Regional Office nearest you (see Section 10, "DIONEX Regional Offices").

6.3.3 LOSS OF FRONT END RESOLUTION

If poor resolutions and efficiencies are observed for the very early eluting peaks near the system void volume compared to the later eluting peaks, check the following:

A. Improper eluent concentration may be the problem. Remake the eluent as required for your application. Ensure that the water and chemicals used are of the required purity.

B. Column overloading may be the problem. Reduce the amount of sample ions being injected onto the analytical column by either diluting the sample or injecting a smaller volume onto the column.

C. Sluggish operation of the injection valve may be the problem. Check the air pressure and make sure there are no gas leaks or partially plugged port faces. Refer to the valve manual for instructions.

D. Improperly swept out volumes anywhere in the system prior to the guard and analytical columns may be the problem. Swap components, one at a time, in the system prior to the analytical column and test for front-end resolution after every system change.

6.4 SPURIOUS PEAKS

A. If the samples contain an appreciable level of aromatic weak acids or fatty acids (larger than 6 carbons) and the column is used with a weak eluent system, these anions may contaminate the analytical column. The retention times for the analytes will then decrease and spurious, inefficient (broad) peaks can show up at unexpected times. Clean the column as indicated in Section 7.4, "Column Cleanup".

B. If you need assistance in determining the best way to clean strongly retained solutes in your specific sample matrix from the IonPac ICE-AS6 columns, contact the DIONEX Regional Office nearest you (see Section 10, "DIONEX Regional Offices").

C. When an injection valve is actuated, the possibility of creating a baseline disturbance exists. This baseline upset can show up as a peak of varying size and shape. It will happen when the injection valve needs to be cleaned or retorqued (see valve manual). Check to see that there are no restrictions in the tubing connected to the valve. Also check the valve port faces for blockage and replace them if necessary. Refer to the Valve Manual for troubleshooting and service procedures. Small baseline disturbances at the beginning or at the end of the chromatogram can be overlooked as long as they do not interfere with the quantification of the peaks of interest.

If cleaning and retorquing the valve does not help, replace the valve. Use a DIONEX High Pressure Injection Valve (P/N 037142) or a DIONEX High Pressure Inert Valve (P/N 037143) as required.

For DX-300 systems equipped with a Rheodyne Microinjection Valve, Model 9126 (DIONEX P/N 044697), consult the accompanying manual for service instructions.

D. When doing trace analysis with solvents in the eluent, a solvent peak will appear around the total exclusion volume of the column. This occurs at 12-15 minutes after injection when operating at a flow rate of 1.0 mL/min. This is a suppressor phenomenon that can be avoided by making the solvent concentration of the sample the same as the solvent concentration of the eluent.

SECTION 7 - COLUMN CARE

7.1 RECOMMENDED OPERATION PRESSURES

Operating a column above its recommended pressure limit can cause irreversible loss of column performance. The maximum recommended operating pressure for IonPac ICE-AS6 columns is 900 psi.

7.2 COLUMN START-UP

The column is shipped in eluent (0.4 mM acid) storage solution.

Prepare the eluent shown on the test chromatogram, install the column in the chromatography module and test the column performance under the conditions described in the test chromatogram. Continue making injections of the test standard until consecutive injections of the standard give reproducible retention times. Equilibration is complete when consecutive injections of the standard give reproducible retention times.

7.3 COLUMN STORAGE

For both short-term storage and long-term storage, eluent (0.4 mM acid) should be used as the storage solution. Flush the column for a minimum of 10 minutes with the storage solution. Cap both ends securely, using the plugs supplied with the column. When putting the column back into service after storing it, pump fresh eluent through the column with the column disconnected from the suppressor for about 20 minutes to clean out any polymer leach from the resin. Polymer leach from the column can severely contaminate the suppressor.

7.4 COLUMN CLEANUP

The following column cleanup protocols have been divided into three general isocratic protocols to remove acid-soluble, base-soluble or organic contaminants. They can be combined into one gradient protocol if desired but the following precautions should be observed.

Always ensure that the cleanup protocol used does not switch between eluents which may create high pressure eluent interface zones in the column. High pressure zones can disrupt the uniformity of the packing of the column bed and irreversibly damage the performance of the column. High pressure zones in the column can be created by pumping successive eluents through the column that are not miscible. The precipitation of the salts in solvents during column rinses can result in very high pressure zones. High viscosity mixing zones can be created between two eluents having solvents with a very high energy of mixing.

When in doubt, always include short column rinse steps to reduce the solvent content of the eluent to 5% levels and the ionic strength of the eluent to 5 mM levels to avoid creating high pressure zones in the column that may disrupt the uniformity of the column packing.

7.5 CHOOSING THE APPROPRIATE CLEANUP SOLUTION

A. Iron contamination of the ICE-AS6 results in a decease in peak heights. However, successive injections of citrate samples will remove the iron resulting in increasing peak heights.

   Citric acid solutions in the concentration range of 2 to 5 mM will remove a variety of metals. If after citric acid treatment, the chromatography still suggests metal contamination, treatment with chelating acids such as oxalic acid is recommended.

B. Organic solvents can be used alone if the contamination is nonionic and hydrophobic. The degree of nonpolar character of the solvent should be increased as the degree of hydrophobicity of the contamination within the range of acceptable solvents listed in Table 3, "HPLC Solvents for Use with IonPac ICE-AS6 Columns".

C. Acid solutions such as 5 to 10 mM HCl can be used with compatible organic solvents to remove contamination that is ionic and hydrophobic. The acid suppresses ionization and ion exchange interactions of the contamination with the resin. The organic solvent then removes the subsequent nonionic and hydrophobic contamination. See Section D above.

A frequently used cleanup solution is 5 mM heptafluorobutyric acid in 10% acetonitrile. This solution must be made immediately before use because the acetonitrile will decompose in the acid solution during long term storage. Regardless of the cleanup solution chosen, use the following cleanup procedure in Section 7.6, "Column Cleanup Procedure", to clean the ICE-AS6.

7.6 COLUMN CLEANUP PROCEDURE

A. Prepare a 500 mL solution of the appropriate cleanup solution using the guidelines in Section 7.5, "Choosing the Appropriate Cleanup Solution".

B. Disconnect the AMMS-ICE from the IonPac ICE-AS6 Analytical Column. Connect the ICE-AS6 directly to the pump. Double check that the eluent flows in the direction designated on the column label. Direct the effluent from the outlet line of the ICE-AS6 to a separate waste container.

C. Set the pump flow rate to 0.50 mL/min.

D. Pump the cleanup solution through the column for 60 minutes.

E. If your cleanup solution contains a solvent between 10 and 15%, use a gradual gradient which reaches a maximum solvent concentration after 30 minutes. Wash the column for 30 minutes and then ramp back down to the eluent over 15 to 30 minutes.

G. Reconnect the AMMS-ICE to the ICE-AS6 Analytical Column and connect the ICE-AS6 to the injection valve.

H. Equilibrate the ICE-AS6 with eluent before resuming normal operation.

SECTION 8 - INSTALLATION OF DIONEX LIQUID LINE FITTINGS

8.1 LIQUID LINE CONVERSION BETWEEN 10-32 AND 1/4-28 FITTINGS

During the course of installing and using the IonPac ICE-AS6 Analytical column, it may be necessary to obtain a transition liquid line for use between a 10-32 eluent port and a component that requires a 1/4-28 liquid line fitting. PEEK tubing, ferrules and bolts should be used for all liquid line connections between 10-32 ports. A Tefzel transition liquid line with a PEEK 10-32 fitting on one end and a 1/4-28 ThermoFlare fitting on the other end can be used in applications with or without solvent where the tubing is not subject to pressures over 2,000 psi. The following Tefzel transition liquid lines can be obtained from DIONEX:

P/N	Description
043780	Tefzel liquid line, 0.007" ID x 12" long with one 10-32 and one 1/4-28 fitting
043781	Tefzel liquid line, 0.012" ID x 12" long with one 10-32 and one 1/4-28 fitting

Reposition the 10-32 threaded fitting bolt (P/N 043275) and a double-cone ferrule (P/N 043276) on the line to make the length of the line between components in the eluent flow path as short as possible.

8.2 ASSEMBLY AND INSTALLATION OF 10-32 PEEK FERRULE/BOLT FITTINGS

The DIONEX Ferrule Fitting System consists of a length of 1/16" OD tubing, a 10-32 threaded tapered port, a HPLC 10-32 threaded fitting bolt (P/N 043275) and a double-cone ferrule (P/N 043276). The fitting is intended to be installed into a 10-32 threaded, tapered port as shown in Figure 24, "10-32 PEEK Ferrule Fitting System", below. The bolt and ferrule are fabricated from high-strength PEEK plastic which conforms to a relatively large range of port geometries but works most reliably in the DIONEX 10-32 PEEK ports. The DIONEX Ferrule Fitting System can be used on any semi-rigid tubing such as Tefzel or rigid tubing such as PEEK or stainless steel tubing with an outside diameter of 0.062". Although stainless steel tubing can be used, it should be avoided because it can damage PEEK ports if the bolt/ferrule assemblies are not properly made and because it will introduce metals into the analytical flow path. As is true for all of the liquid lines in the eluent flow path, an internal diameter of 0.012" or less is required (0.010" or 0.005" ID PEEK tubing should be used on 10-32 columns and components).

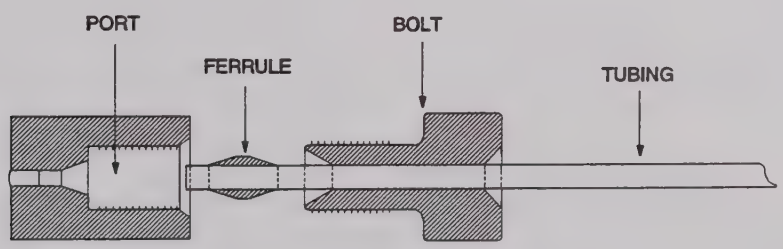


Figure 24

10-32 PEEK Ferrule Fitting System

The following procedure is used to install the 10-32 PEEK bolt and ferrule fittings on liquid lines. For the most reliable performance, DIONEX recommends the use of PEEK tubing whenever liquid lines with 10-32 fittings are required. Whenever a transition liquid line to a component with a 1/4-28 fitting is required, a Tefzel transition liquid line with a PEEK 10-32 ferrule/bolt fitting on one end and a 1/4-28 ThermoFlare fitting on the other end can be used.

A. The PEEK tubing provided by DIONEX has a 0.062" OD which is important so that the ferrule fits snugly on the tubing and thus makes a good seal. In situations where Tefzel tubing will be used, the PEEK bolt and ferrule do not easily slide onto Tefzel tubing when making a transition liquid line, a short section of the Tefzel tubing should be stretched slightly to reduce its diameter (see Figure 25 for details on assembling a Tefzel transition liquid line with a PEEK bolt and ferrule).

B. Slide the end of the tubing through the hex-end of the PEEK bolt and then through the ferrule. The ferrule is symmetrical and can be pushed onto the tubing through either end.

C. Pull the tubing through the 10-32 PEEK bolt and the ferrule until the ferrule fits snugly on a section of the PEEK tubing or if Tefzel tubing is used, pull the ferrule onto the unstretched section of Tefzel tubing.

D. Cut the tubing off at least 1/8" past the end of the ferrule. Make sure that the end is cut off carefully at a right angle to the length of the tubing and that there are no nicks or burrs on the end of the tubing. Being careful while making liquid line fittings will reduce dead volumes and band broadening. This is important for maximum system performance.

E. Place the tubing with the ferrule and the 10-32 PEEK bolt into the 10-32 port and push the tubing through the bolt until it fits snugly against the bottom of the port. Making sure that there is no gap between the end of the tubing and the bottom of the port will ensure low dead volumes and minimal band broadening. This is important for maximum system performance.

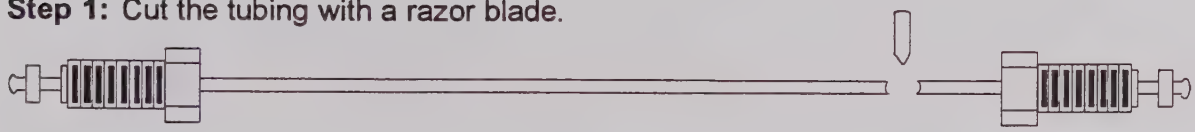
CAUTION

Tighten the 10-32 PEEK bolt by hand until it is fingertight. As the bolt is tightened further, the ferrule will compress tightly around the tubing. In the case of Tefzel tubing, the compression of the ferrule will also compress the semi-rigid Tefzel tubing. Overtightening the bolts can reduce the ID of Tefzel tubing until flow through the tubing is completely restricted. Severe overtightening of the bolt in all cases can damage the mating surfaces of the 10-32 port. Fingertight torquing of the liquid line bolts provides leak free operation up to 3,000 psi.

F. For the best performance, end fittings should be mated to ports. Once a ferrule has been compressed inside a particular 10-32 port, it can be removed and reinstalled in the same port without sacrificing performance. If a liquid line needs to be routed to a new port, a new ferrule should be mounted on the end of the line and compressed in the new port to form a mated ferrule/port pair. This ensures the best sealing properties.

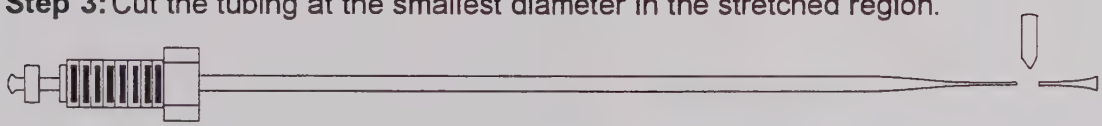
Tefzel Tubing with two 1/4-28 ThermoFlare/bolt assemblies

Step 1: Cut the tubing with a razor blade.

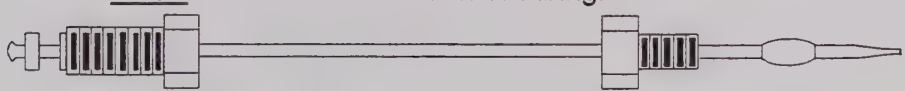


Step 2: Stretch the tubing only if it does not slip through the ferrule.

Step 3: Cut the tubing at the smallest diameter in the stretched region.



Step 4: Slip a 10-32 PEEK bolt and ferrule on the stretched tubing and then pull them PAST the stretched section of tubing.



Step 5: Cut the tubing perpendicular to its axis and approximately 1/8" from ferrule. Remove ALL of the stretched tubing!

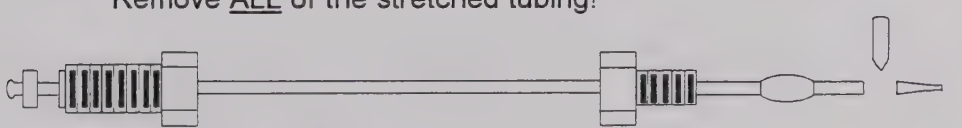


Figure 25

Tighten the 10-32 ferrule/bolt assembly fingertight ONLY.
Overtightening will constrict the Tefzel tubing

8.3 ASSEMBLY AND INSTALLATION OF THERMOFLARE FITTINGS (ON TEFZEL TUBING ONLY)

If you already have a short connecting liquid line with ThermoFlare fittings on both ends for use between components with 1/4-28 ThermoFlare end fittings, you may choose to cut off one of the 1/4-28 ThermoFlare end fittings with a razor blade and replace it with a 10-32 PEEK ferrule/bolt assembly, as described in Section 8.2, "Assembly and Installation of 10-32 PEEK Ferrule/Bolt Fittings".

If you choose to assemble new Tefzel lines with 1/4-28 end fittings, the sealing surface of the 1/4-28 ThermoFlare fitting is made by first heating and then flaring the end of the tubing with a special flaring tool (120 VAC version, P/N 037196 or 230 VAC version, P/N 037197). Complete instructions for using the tool are in Document No. 032843.

If you prefer not to buy a flaring tool, liquid lines designed for use in the eluent flow path with factory-made ThermoFlares are available from DIONEX in the following lengths:

P/N	Description
039335	Tefzel line, 0.012" ID x 4" long with two 1/4-28 fittings
039336	Tefzel line, 0.012" ID x 8" long with two 1/4-28 fittings
039337	Tefzel line, 0.012" ID x 12" long with two 1/4-28 fittings
039338	Tefzel line, 0.012" ID x 24" long with two 1/4-28 fittings

Column Accessories

048238	Bed Support Assembly, 9mm Columns with 10-32 Fittings
048297	Zitex Bed Support, 9mm Columns with 10-32 Fittings
048298	End Fitting, 9mm Columns with 10-32 Fittings
043780	Tefzel line, 0.007" ID x 12" long with one 10-32 and one 1/4-28 fitting
043781	Tefzel line, 0.012" ID x 12" long with one 10-32 and one 1/4-28 fitting
039335	Tefzel line, 0.012" ID x 4" long with two 1/4-28 fittings
039336	Tefzel line, 0.012" ID x 8" long with two 1/4-28 fittings
039337	Tefzel line, 0.012" ID x 12" long with two 1/4-28 fittings
039338	Tefzel line, 0.012" ID x 24" long with two 1/4-28 fittings
042690	PEEK Tubing, 0.010" ID x 0.062" OD
044221	PEEK Tubing, 0.005" ID x 0.062" OD
043275	Bolt, Double Cone Ferrule Fitting, 10-32 (PEEK)
043276	Double Cone Ferrule, 10-32 (PEEK), ea
043225	Double Cone Ferrule, 10-32 (PEEK), pkg of 10
042772	Plug, Ferrule Fitting, 10-32 (PEEK), ea
042806	Union, Black PEEK 10-32 ferrule to 1/4-28 ThermoFlare Tefzel Line
039296	Union, 1/4-28 to 1/4-28, 0.020" ID
039056	Union, 1/4-28 to 1/4-28, 0.062" ID
037196	ThermoFlare Tool (115 V)
037197	ThermoFlare Tool (230 V)
041620	ThermoFlare Tubing Kit
039302	ThermoFlare Washers
044697	Rheodyne Microinjection Valve, Model No. 9126

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IonPac ICE-AS6 Analytical Column
INSTALLATION INSTRUCTIONS AND TROUBLESHOOTING GUIDE

Your opinions and comments help us produce better manuals. Please answer the following questions by circling a number from 1 to 4.

Is the manual easy to understand? **Yes ← 4 3 2 1 → No**

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_____

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_____

Is the manual accurate? **Yes ← 4 3 2 1 → No**

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Is the manual complete? **Yes ← 4 3 2 1 → No**

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_____

Use the space below to suggest changes that you feel would improve the manual. Please use page and section references when possible. Attach extra sheets if necessary.

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IonPac® ICE-AS6 QuickStart

Read This First !

1. Make the required stock and working solutions for eluents. See Section 4, "Operation", for details. Note operation precautions and chemical purity requirements.

2. Run the Production Test Chromatogram as a system check. See Section 5.3, "Production Test Chromatogram", for details.

3. See Section 7, "Column Care", for column cleanup and long-term storage recommendations.

DIONEX

IONPAC® ICE-AS6 ANALYTICAL COLUMN (P/N 046023) (10-32) TEST CHROMATOGRAM

This chromatogram was obtained during a quality control test. Please note that test fixtures were used, so slight variations from the test chromatogram may occur on your instrument. Eluent batch and laboratory climate can also affect chromatographic performance.

Eluent: 0.4 mM Heptafluorobutyric acid

Flow Rate: 1.0 mL/min

Detection: Suppressed Conductivity at 10 µSFS

Standard: 50 µL of:

	Acid	Concentration
1.	Oxalic	5.0 mg/L
2.	Tartaric	10.0 mg/L
3.	Citric	15.0 mg/L
4.	Malic	20.0 mg/L
5.	Glycolic	10.0 mg/L
6.	Formic	10.0 mg/L
7.	Lactic	10.0 mg/L
8.	Hydroxyisobutyric	30.0 mg/L
9.	Acetic	25.0 mg/L
10.	Succinic	40.0 mg/L

Where 1 mg/L = 1 ppm

Storage Solution: Eluent

Serial No. 1330 **Pressure (PSI)** 420 **Date** 7-19-95

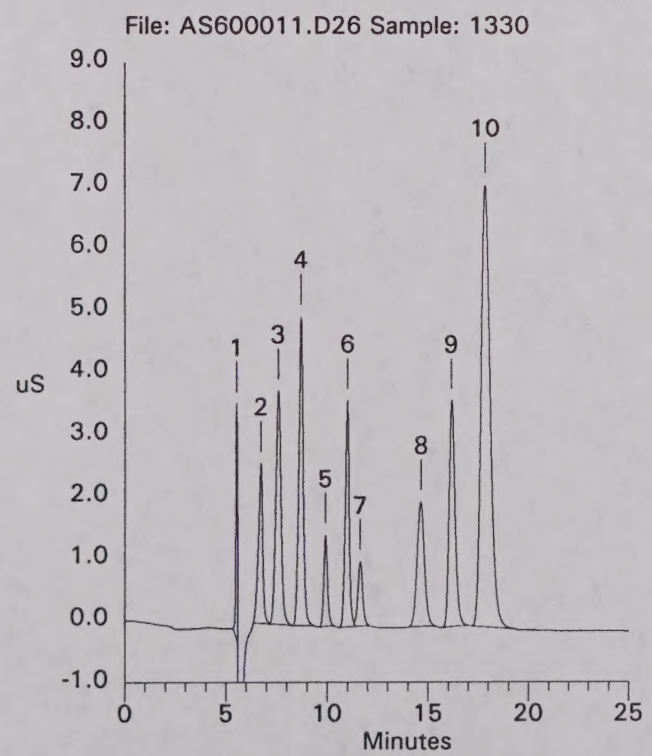
Dionex Corporation
IonPac ICE-AS6 Analytical Column
Test Chromatogram

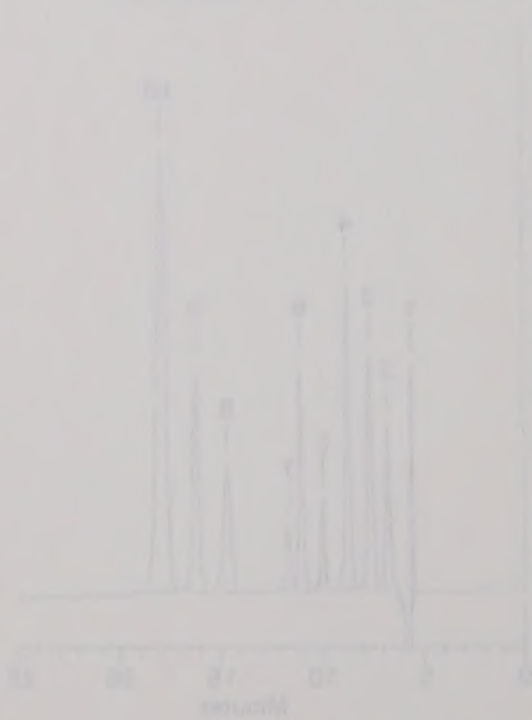
Sample Name: 1330	Date: 07/19/1995 17:49:44
Data File : C:\DX\DATA\ICE_AS6\AS600011.D26	
Method : C:\DX\METHOD\ICE_AS6\ICE6_4.MET	
ACI Address: 2 System: 2 Inject#: 26	Detector:CDM-1
Analyst : Column:	

********** Component Report: All Components **********

Pk. Num	Ret Time	Component Name		Efficiency	Asymmetry	Resolution
1	5.52	Oxalic	5 ppm	22447	0.9	4.71
2	6.72	Tartaric	10 ppm	5492	0.7	2.08
3	7.58	Citric	15 ppm	4162	1.0	2.65
4	8.72	Malic	20 ppm	8193	0.8	3.36
5	9.92	Glycolic	10 ppm	14576	1.3	3.21
6	11.00	Formic	10 ppm	16214	0.9	1.64
7	11.63	Lactic	10 ppm	11996	1.1	5.49
8	14.63	HIBA	30 ppm	7668	1.1	2.55
9	16.17	Acetic	25 ppm	14748	1.5	2.46
10	17.83	Succinic	40 ppm	7542	1.4	0.00

Totals





MP MASTER
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